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THE INTERNAL TEMPERATURES OF FRUIT TREE BUDS

III. TRIALS WITH HUMIDIFIED HEAT FOR THE CONTROL OF FROST DAMAGE

By JOHN GRAINGER

Tolson Memorial Museum, Ravensknowle, Huddersfield

With 4 Text-figures

GRAINGER (1939) discussed the principles of the most economical control of frosts in orchards. Flame-type heaters which burn crude oil raised the temperature of the air by 2–3° C., but the internal temperature of an apple bud in the heated air was lowered by about 1° C., owing to the increased evaporation. A method of heating which maintained, or increased, the relative humidity of the air within the orchard should therefore prove more economical than the flame-type heater, since it would prevent the lowering of the internal temperature of the bud. An investigation was planned to find such an improved method of control. The distribution of heat and relative humidity from flame-type heaters, and from "smudging" fires of partly dry garden refuse was studied, and then methods of humidifying the heat from the flame-type heaters. Finally, a simple method of burning crude oil, in conjunction with wet, long litter, as grass, straw or lawn clippings, was evolved. It appears to provide a net result, so far as the internal temperature of the apple bud is concerned, equal to the flame-type heaters, with greatly reduced fuel consumption.

THE DISTRIBUTION OF HEAT AND RELATIVE HUMIDITY FROM A "SMUDGING" FIRE

A large, smoky fire ("smudging" fire) was made with moist garden refuse on 16 November 1938, when a light south-south-east wind was blowing at about 1 m.p.h. Temperature and humidity readings, by mercury thermometer and spinning hygrometer respectively, were made before the fire was lit, and during the period of its burning. Isotherms based upon

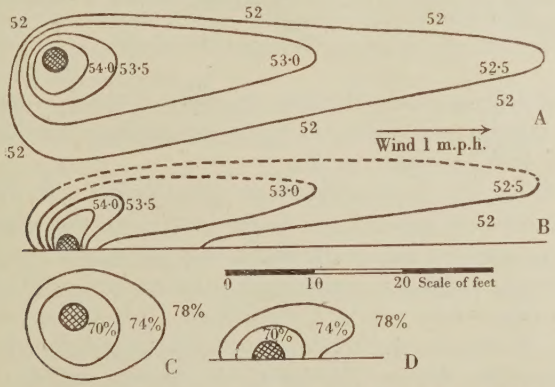


Fig. 1. A and B, isotherms showing the heating effect of a "smudging" fire, 16 November 1938. Temperature of the orchard before heating, 52° F. A in plan, B in vertical section. C and D, zones of humidity round the same fire. C in plan, D in vertical section.

30 readings are given in Fig. 1, and show that the fire warmed the air to the extent of $\frac{1}{2}$° F. at a distance of 55 ft. from the fire, but that at 7 ft. the temperature was only $1\frac{1}{2}$° F. above that of the unheated garden. The relative humidity was not markedly lowered (Fig. 1 C, D), and except within a few feet of the fire, was not diminished by more than 8%, from 78 to 70%. This is apparently the kind of heating required in frost control, but it was deemed that, for the result obtained, the method required too much material and attention.

The distribution of heat and relative humidity from flame-type, oil-burning heaters

Similar measurements of temperature and relative humidity around single flame-type crude-oil heaters were made upon three occasions in the autumn and winter of 1938. Figs. 2 and 3 portray the maximum vertical and lateral distribution of temperatures, recorded as in

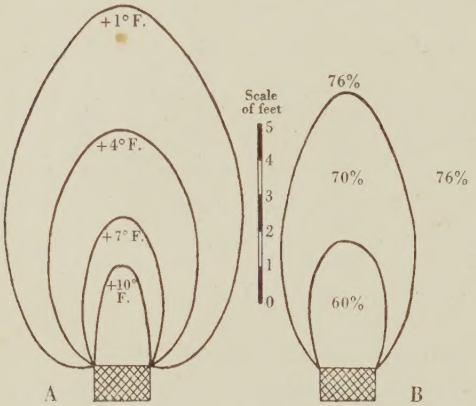


Fig. 2.

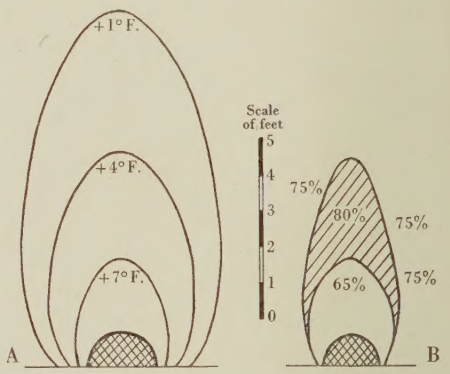


Fig. 3.

Fig. 2. A, isotherms showing the heating effect of an oil-burning, flame-type heater 3 December 1938. Temperature of the orchard before heating 35° F. B, zones of relative humidity for the same heater.

Fig. 3. A, isotherms showing the warming effect of humidified heating, 3 January 1939. Temperature of the orchard before heating 34° F. B, zones of relative humidity for the same method of heating, namely, eight oil balls humidified with saturated lawn clippings and long grass.

still air. A slight wind deflects the distribution obliquely, whilst a stronger wind minimizes it considerably. The distributions shown in the figures appear to hold good, whether the air before heating was above or below 32° F.

Isotherms for an oil-burning flaming heater are given in Fig. 2A. They show that considerable rise in temperature is effected near to the heater, but the warming action rapidly falls off, and 5 ft. away from the source the rise is only about 4° F. (2° C.). This confirms previous results (Grainger, 1939) that temperatures near an apple bud 5 ft. away from such a heater were seldom raised more than 2° C. in still air. It would appear that the effective distribution of heated air amongst the trees of an orchard depends largely upon the directive effect of slight, downwardly flowing (katabatic) air currents, which occur upon sloping ground during a radiation frost.

The relative humidity is also markedly lowered within a considerable volume of air near

the flame-type heater (Fig. 2B). The effect of this dry air in lowering bud temperatures through increased evaporation has already been discussed (Grainger, 1939) and is shown in Fig. 4 from 8.15 to 8.40 p.m.; the lowering is here very marked.

A NEW METHOD OF ORCHARD HEATING

Blocks of various absorbent materials placed round the ordinary flame-type heaters did not appear to set free sufficient water vapour to affect the relative humidity of the atmosphere. The absorption of crude oil into balls of papier maché was then tried as a method of heating. Newspapers were torn and pulped in water, squeezed into balls between the hands, and were then dried thoroughly at 65° C. The balls were each about $2\frac{1}{2}$ in. in diameter, and when immersed in oil, absorbed nearly 100 c.c. They were used in groups of six or eight, eight balls containing nearly $1\frac{1}{2}$ pints of oil which, in this form, can be ignited readily with a match. The heating effect of a group of such balls is very similar to that of the flame-type heater shown in Fig. 2.

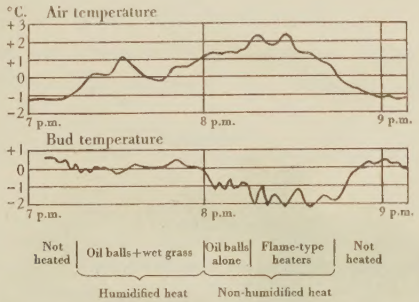


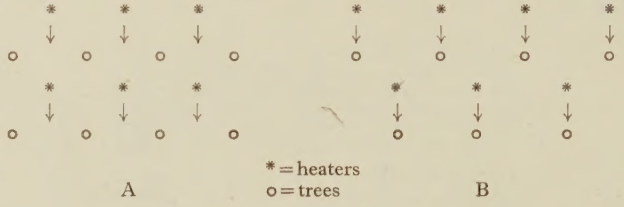
Fig. 4. 3 January 1939. Air temperatures and bud temperatures during various methods of orchard heating. Air temperatures are recorded on a bi-metallic thermograph, and represent actual values; bud temperatures are expressed in relation to the surrounding air, and are measured electrically by the method described by Grainger (1939).

Similar balls, or rectangular blocks of papier maché, which had been steeped in water, were first used to provide the necessary water vapour. They were placed above the burning balls, but they, again, did not appear to yield sufficient water vapour to the heated air. Adequate humidification was finally obtained by placing a large forkful of thoroughly soaked, long garden refuse, such as grass or lawn mowings, over a heap of eight burning balls, and the fire then burned without attention for more than 2 hr. The saturated refuse did not burn away, and the tenacious burning of the oil balls maintained the heating effect. The fire gave off copious steamy smoke, and Fig. 3B shows that it *raised* the relative humidity in a region a little above the fire. More important still was its effect upon the bud temperature. This was not depressed (Fig. 4, 7.20–8 p.m.) as with the flaming heater (Fig. 4, 8.15–8.40 p.m.). These effects, as graphically combined in the consecutive record of Fig. 4, are typical of six records for the humidified heating, and of 21 records for the flame-type heaters.

Figs. 3A and 4 show that the humidified heating does not warm the air to such a degree as the oil-flame heaters (Figs. 2A, 4), but neither does it reduce the temperature within the apple bud. The net result, so far as the internal temperature of the bud is concerned, is much the same in both cases, but the humidified heat is accomplished with an expenditure

of approximately $1\frac{1}{2}$ pints of oil, whereas the flaming heater used $4\frac{3}{4}$ pints for a similar period of 2 hr. The humidified heating also has the advantage that it controls the frost at its source, the cloud of smoke and steam which it produces insulating the ground, and minimizing radiation from the earth to an open sky.

It has been pointed out (Grainger, 1939) that there is not much lateral distribution of warmth from a heater, and this is emphasized by the distributions shown in Figs. 2 and 3. The disposition of trees upon slightly sloping ground could either hinder or help a proper distribution of heat to the trees. Downwardly flowing (katabatic) winds would here arise of necessity during a radiation frost. The usual diagonal arrangement of heaters between trees planted on the square (A) upon a slope downwards in the direction of the arrows, would give the warmest parts of the orchard *between* the rows of trees. Planting the trees alternately as in B would allow a disposition of heaters immediately above them on the slope, and the warmest parts of the orchard would be in the lines of trees.



Summary

An account is given of the distributions of heat and relative humidity from three methods of orchard heating, together with the results of experiments with a simple type of humidified heating. Ignited balls of papier maché which had been soaked in crude oil, were humidified with saturated garden refuse, such as long grass or lawn clippings. This arrangement heats the air, and at the same time either maintains or enhances the relative humidity. No lowering of the internal temperature of the bud is produced, and the method helps to control a frost at its source, by diminishing radiation of heat from the earth to an open sky. Frosts may therefore be controlled in practice by the expenditure of about one-third the fuel used in the flame-type heaters.

Lateral distribution of heat from orchard heaters is not great. Suggestions are made for the alternate disposition of fruit trees and heaters upon a slope. Advantage may thereby be taken of the katabatic wind which occurs during a radiation frost, to direct the heated air towards the trees.

The writer wishes to express his thanks to Messrs Geo. Monro, Ltd., for their continued interest in the problem, and for their kind provision of orchard heaters for the experiments.

REFERENCE

Grainger, J. (1939). The internal temperatures of fruit tree buds. II. *Ann. appl. Biol.* **26**, 1–13.

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ASSOCIATION OF APPLIED BIOLOGISTS

IMPORTANT NOTICE

At a meeting of the Council of the Association on 15 September 1939, it was decided to make every effort to continue publication of the *Annals of Applied Biology* during the emergency period. It was apparent that certain economies would be necessary in order to meet increased costs of production, a shortage of paper, and possibly a diminished income. These needs have partially been met by a change in the format of the *Annals*, which is exemplified in the present issue. This part of 160 pages contains approximately the same amount of matter as previous parts of about 215–220 pages.

It is hoped that Members will help by:

(*a*) condensing their manuscripts to the utmost—the maximum number of new ideas and data in the minimum number of words;

(*b*) eliminating all unnecessary text-figures, plates, tables, graphs, etc., and ensuring that such as are necessary are arranged and grouped in the most economical way;

(*c*) reading carefully the "Notice to Contributors" on the back cover of the *Annals*.

It has been found necessary to make the following arrangements relating to the communication of papers to the Association and for publication in the *Annals of Applied Biology*:

1. The author of every paper submitted to the Association or for publication in the *Annals* is requested to sign a declaration that, to the best of his knowledge, the paper does not contain any information that contravenes the Official Secrets Act and to send the declaration with his paper.

2. An author directly employed by any Government Department or similar body associated with the prosecution of the war, or whose work submitted for publication relates to such department or body, is requested to send with his paper a written statement from such department or body to the effect that he has received authority to submit the paper for publication by the Association.

The continuation of the *Annals* depends upon (*a*) a steady supply of manuscripts for publication, and (*b*) the maintenance of subscriptions to the Association. Will Members please do their utmost to ensure that both these needs are met. Subscriptions are due on 1 January of each year and it is most helpful if they are paid promptly.

Finally, owing to difficulties inevitable in these disturbed times, it is imperative that all correspondence relating to the *Annals* be dealt with as promptly as possible—delay in the return of a proof by one author may dislocate seriously the entire production of the *Annals*.

THE NUTRITION OF LETTUCES GROWN AS SAND CULTURES UNDER GLASS

By R. M. WOODMAN

Horticultural Research Station, Cambridge University

THE effects of variations in the supplies of nitrogen, phosphorus and potassium on the appearance, growth and yield of the tinted lettuce May King have been studied by Woodman (1936, 1939 *a*, *b*, *c*, *d*). The present experiments were designed: (i) to test the main findings of the previous experiments in a comprehensive experiment, (ii) to discover how far certain deficiency symptoms noticed in May King were common to a non-tinted lettuce, and, (iii) and (iv), to ascertain what effects variations in the supply of calcium and magnesium respectively had on sand cultures of lettuce.

COMPREHENSIVE EXPERIMENT WITH MAY KING LETTUCE

The "lay-out" was a randomized-block arrangement of thirty-nine replications of five treatments with nutrient solutions A, B, C, D, and E, 195 cultures in all. The concentrations in p.p.m. of certain elements in the solutions are given in Table 1, where the divergences from the control solution A are italicized. The salts used in preparing the solutions have been described (Woodman, 1936, 1939 *a*). The seed was sown on 14 December 1936, in the actual plant pots (each containing about 13 lb. of the sand) used as culture jars, in order to avoid transplanting, and so that germination and growth might take place in the desired medium. Germination occurred in every pot by 22 December, and the seedlings were thinned to leave one per pot on 24 December. The harvest was on 9 April 1937. Each culture, during the experiment, received 10·5 l. of its particular nutrient solution, usually in lots of 250 c.c. every 3 days.

Throughout the experiment the greenhouse was maintained at 47–53° F. The daily averages of sunshine in hours were: 1st week, commencing 19 December 1936, 2·7; 2nd week, 1·8; 3rd, 2·4; 4th, 1·3; 5th, 2·6; 6th, 0·4; 7th, 1·6; 8th, 3·7; 9th, 0·4; 10th, 3·4; 11th, 1·5; 12th, 2·1; 13th, 3·3; 14th, 5·1; 15th, 4·9; and 16th, ending on the day of harvest, 9 April 1937, 2·4.

Summary of observations made on the cultures. At first there was little difference in tint, but, towards harvest, traces and small patches of reddish purple developed, particularly with *E*, where phosphorus and nitrogen were both low, and to a lesser extent with *D*, where the supply of phosphorus only was lower than for the control. The extent and intensity of this tinting was in the following descending order: E > D > A = B = C. This bore out, as far as was possible in winter and early spring, results previously obtained, where it was demonstrated that lack of phosphorus or nitrogen resulted in tinted lettuces with May King (Woodman, 1936, 1939 *a*, *b*). Solution E yielded plants of the lightest green, despite this tendency to tinting.

Solutions A, B, and C, all gave larger lettuces than D (with less phosphorus), and these in turn were larger than with solution E (less phosphorus and nitrogen); but lettuces with A, B, and C, appeared to be equal in size despite the large differences in the amounts of potassium supplied to the cultures. The observations thus fully bear out what has previously been noted in detailed experiments (Woodman, 1936, 1939 *a*, *b*, *c*, *d*).

At harvest the numbers of lettuces at an advanced stage of hearting for each treatment of

TABLE 1

Nutrient solution used	N p.p.m.	P p.p.m.	K p.p.m.	Ca p.p.m.	Mg p.p.m.	Fe p.p.m.	S p.p.m.	Na p.p.m.
(i) May King experiment								
A	32·96	21·84	22·44	9·03	5·05	0·50	16·15	86·56
B	32·96	21·84	*44·88*	9·03	5·05	0·50	*25·35*	86·56
C	32·96	21·84	*89·76*	9·03	5·05	0·50	*43·75*	86·56
D	32·96	*10·92*	22·44	9·03	5·05	0·50	16·15	*70·36*
E	*16·48*	*10·92*	22·44	9·03	5·05	0·50	16·15	*43·28*
(ii) Cheshunt Early Giant experiment								
A/N	*32·96*	21·84	22·44	9·03	5·05	0·50	16·15	*86·56*
B/NPK	16·48	21·84	22·44	9·03	5·05	0·50	16·15	59·48
C/N	*6·18*	21·84	22·44	9·03	5·05	0·50	16·15	*42·55*
D/N	*2·06*	21·84	22·44	9·03	5·05	0·50	16·15	*35·78*
E/N	*0·41*	21·84	22·44	9·03	5·05	0·50	16·15	*33·08*
G/N	Nil	21·84	22·44	9·03	5·05	0·50	16·15	*32·40*
A/P	16·48	*43·68*	22·44	9·03	5·05	0·50	16·15	*91·88*
B/NPK	16·48	21·84	22·44	9·03	5·05	0·50	16·15	59·48
C/P	16·48	*8·19*	22·44	9·03	5·05	0·50	16·15	*39·23*
D/P	16·48	*2·73*	22·44	9·03	5·05	0·50	16·15	*31·13*
E/P	16·48	*1·36*	22·44	9·03	5·05	0·50	16·15	*29·10*
F/P	16·48	*0·27*	22·44	9·03	5·05	0·50	16·15	*27·49*
G/P	16·48	Nil	22·44	9·03	5·05	0·50	16·15	*27·08*
A/K	16·48	21·84	*44·88*	9·03	5·05	0·50	*25·35*	59·48
B/NPK	16·48	21·84	22·44	9·03	5·05	0·50	16·15	59·48
C/K	16·48	21·84	*11·22*	9·03	5·05	0·50	*11·55*	59·48
D/K	16·48	21·84	*5·61*	9·03	5·05	0·50	*9·25*	59·48
E/K	16·48	21·84	*2·81*	9·03	5·05	0·50	*8·10*	59·48
F/K	16·48	21·84	*0·56*	9·03	5·05	0·50	*7·18*	59·48
G/K	16·48	21·84	Nil	9·03	5·05	0·50	*6·95*	59·48
(iii) Ca experiment								
A/Ca	32·96	21·84	22·44	9·03	5·05	0·50	16·15	86·56
B/Ca	32·96	21·84	22·44	*4·52*	5·05	0·50	16·15	86·56
C/Ca	32·96	21·84	22·44	*2·26*	5·05	0·50	16·15	86·56
D/Ca	32·96	21·84	22·44	*0·45*	5·05	0·50	16·15	86·56
(iv) Mg experiment								
A/Mg	32·96	21·84	22·44	9·03	5·05	0·50	16·15	86·56
B/Mg	32·96	21·84	22·44	9·03	*2·53*	0·50	*12·82*	86·56
C/Mg	32·96	21·84	22·44	9·03	*1·26*	0·50	*11·16*	86·56
D/Mg	32·96	21·84	22·44	9·03	*0·25*	0·50	*9·82*	86·56
E/Mg	32·96	21·84	22·44	9·03	*0·13*	0·50	*9·66*	86·56

thirty-nine plants (the figures in parentheses denote additional lettuces which were fully hearted) were: with solution A, 38 (1); B, 37 (1); C, 34 (4); D, 24 (0); and with E, 0 (0). It was obvious that a reduction in the supply of phosphorus, as with D, retarded maturity, and that a simultaneous reduction of nitrogen as well, as in E, still further retarded it, but that alteration in the supply of potassium over a wide range, as with A, B, and C, had little effect in this regard. These results also bore out previous work (Woodman, 1936, 1939*a*, *b*, *c*, *d*).

The yields, etc., with different treatments. The values obtained for each culture were the fresh weights in grams of the top (cut off at the junction with the stalk), root, and whole plant, the corresponding dry weights, the top/root ratios for fresh and dry weights, and the percentage moisture contents of the fresh material of the top, root, and whole plant.

Actual data obtained were too bulky to be reproduced, but are available for examination in this laboratory. The analyses of variance (Wishart & Sanders, 1935) were worked out for the eleven values mentioned above. The corresponding summaries of results are tabulated

TABLE 2. *Summaries of results for comprehensive experiment on May King lettuce*

Description of data		Treatment mean for A	B	C	D	E	General mean	S.E.
Tops, FW	SSS	62·19	60·02	61·15	47·80	22·57	50·75	1·4750
				A = B = C > D > E				
Roots, FW	SSS	17·28	17·36	20·23	14·94	15·74	17·11	0·6515
				C > A = B > D;	A = B = E;	D = E		
Whole plants, FW	SSS	79·47	77·38	81·38	62·74	38·31	67·86	1·6780
				A = B = C > D > E				
Tops, DW	SSS	4·07	3·90	3·69	3·75	2·28	3·546	0·0970
			A > C = D > E;	A = B;	B = C = D > E			
Roots, DW	SS	1·95	1·87	1·91	1·59	1·60	1·785	0·0898
				A = B = C > D = E				
Whole plants, DW	SSS	6·02	5·77	5·60	5·34	3·88	5·331	0·1550
			A = B = C > E;	A > D > E;	B = C = D			
Top/root, FW	SSS	3·67	3·61	3·13	3·29	1·47	3·034	0·0803
				A = B > C = D > E				
Top/root, DW	SSS	2·29	2·42	2·04	2·42	1·47	2·128	0·0965
			A = B = D > E;	B = D > C > E;	A = C			
Tops, % moisture	SSS	93·44	93·33	93·97	92·28	89·93	92·59	0·2935
				A = B = C > D > E				
Roots, % moisture	SS	88·84	89·40	90·46	89·32	89·76	89·56	0·2846
			A = B = D = E;	C > A = B = D;	C = E			
Whole plants, % moisture	SSS	92·43	92·43	92·90	91·49	89·86	91·83	0·1600
				A = B = C > D > E				

in Table 2, together with standard errors of the treatment means. Arithmetical means have been used when dealing with ratios and percentages.

In the first column of Table 2 (or 3, 4, or 5), "FW" and "DW" denote fresh and dry weights. There also occurs here, for each summary of results, one of the descriptions S, SS, SSS, or NS, which indicates that the treatment means were found to be significant in the analyses of variance by the z test at the 5, 1, and 0·1 %, levels, or were not significant, respectively. The treatment means are recorded under the "Treatment mean for" column subheadings, A, B, C, D, and E. The mean of all the results for a subtable is given in the column headed "General mean"; the standard errors of these treatment means are recorded under "S.E." In the second line of each subtable, where z is significant, is given a comparison of significance carried out on the treatment means; the signs "=" and ">" are to be read as "not significantly different from", and "significantly greater than", respectively.

The fresh weights of the lettuces themselves and the fresh and dry weights of the whole plants were not significantly altered over a wide range of concentrations of potash, for A = B = C. The dry matter of the tops was also not influenced to any great extent, as B = C = D; and, though A was just significantly greater than C = D, it was still equal to B. With the fresh roots there was a small significant increase with C over A and B, though A = B; with the dried roots, A = B = C. It would appear, therefore, that the increase of potassium had very little effect on yield.

A decrease in the phosphorus (D) caused significant decreases in the yields of fresh tops, roots, and whole plants below those for A, B, and C. The dry matter of the roots was also

significantly reduced, and the dry matters of the tops and whole plants were equal to those with B and C, but less than those with A.

Reduction in nitrogen (E) caused a further significant decrease in the yield of heads and whole plants (D > E), though the roots were equal (D = E). Similar alterations occurred for the corresponding dry weights. It is evident that nitrogen, as well as phosphorus, is largely concerned in yield.

The moisture contents for tops and whole plants were in the order A = B = C > D > E; but with the roots those for C (most potassium) were significantly increased above all others except those with treatment E. The top/root ratios for both fresh and dry weights with medium E were definitely the lowest.

Experiment with Cheshunt Early Giant lettuce

This was the lettuce used as the non-tinted variety in the second experiment. The experiment included variations of nitrogen, phosphorus, and potassium, in turn. There were eighteen nutrient solutions (Table 1). Three of these, G/N, G/P, and G/K, where N, P, and K were absent in turn, were each applied to three cultures only, and these nine cultures were left outside the arrangement of twelve replications of a randomized block of fifteen treatments (180 cultures in all) which comprised the main portion of the experiment. Solution B/NPK did not, as might appear from Table 1, take part as three treatments, but was the common control to the whole experiment, and was repeated in the N, P, and K portions of Table 1, section (ii), merely for reference purposes. It will be noted that the fact that there is a common control allowed of an extra solution, respectively F/P and F/K, in two of the sections, P and K. There was no solution F/N.

The details were similar to those for the last experiment. The seed (provided by Dr W. F. Bewley of Cheshunt) was sown on 18 September 1936; germination occurred by the 22nd, and the lettuces were singled to one per pot on the 23rd. The harvest was 17 November 1936. During the experiment each culture received 7·5 l. of the appropriate medium, usually in 250 c.c. lots every 2 days. The temperature of the greenhouse was 47–70° F. The daily averages of hours of sunshine during the period were: 1st week, commencing 23 September 1936, 2·9; 2nd week, 5·1; 3rd, 2·3; 4th, 3·9; 5th, 3·2; 6th, 2·7; 7th, 3·1; and 8th, ending with the day of harvest, 17 November 1936, 1·3.

Summary of observations made on the cultures. Nitrogen portion of the experiment. By 16 October 1936, cultures with D/N, E/N, and G/N, were a lighter green than the others. On 28 October, the order of depth of shade of green was: A/N = B/NPK > C/N > D/N > E/N = G/N; this order then held throughout the rest of the experiment, although there was a tendency to a gradual and slight darkening of the plants of lighter shade as they approached maturity. The green of cultures receiving solutions with less phosphorus or potassium than the common control, B/NPK, in the phosphorus and potassium portions of the experiment, was always much darker than with the corresponding treatments for nitrogen, C/N to G/N. There was no trace of red or purple tinting with any treatment (N, P, or K) during the experiment, so that the purple flushes and tints noted with May King lettuce as deficiency symptoms were evidently characteristic of a tinted lettuce (Woodman, 1936, 1939 *a*, *b*).

With a deficiency of nitrogen, starting at C/N, there developed a non-crinkled plant of very even, flat-leaved, regular appearance. A few of the cultures with C/N, and all those receiving solutions with greater deficiencies of nitrogen, developed a characteristic "smooth" appearance, which was not noticed even with non-crinkled plants in the phosphorus and potassium parts of the experiment; this abnormal "smoothness" appeared, therefore, to be characteristic of deficiency of nitrogen with this lettuce.

The linear dimensions (average greatest breadth, and average breadth at right angles to this) of the lettuces in cm. at harvest were: A/N, $15{\cdot}5 \times 14{\cdot}3$; B/NPK, $11{\cdot}7 \times 10{\cdot}5$; C/N, $9{\cdot}5 \times 8{\cdot}8$; D/N, $7{\cdot}3 \times 6{\cdot}5$; E/N, $4{\cdot}8 \times 3{\cdot}8$; and G/N (no nitrogen; three cultures only), 5×4. This was the relative position throughout the experiment, and the lettuces in general were smaller than cultures of May King grown alongside during the same period. A better notion of size can, however, be obtained from the tabulated summaries of results.

At harvest one of treatment A/N was fully hearted, and the other eleven showed definite signs of hearting; with the control, B/NPK, seven showed definite signs of hearting; no lettuce of any other treatment was hearted, or showed signs of hearting. A good supply of nitrogen, therefore, evidently tended to earlier maturity, as with May King (Woodman, 1936, 1939 *b*).

The roots were found at harvest to be normal, pale amber tap roots, which were progressively smaller in size, and possessed of smaller amounts of attached fibre, as the nitrogen progressively decreased.

Phosphorus portion of the experiment. With treatments with less phosphorus than the common control, B/NPK, the green was always much darker than for the corresponding nitrogen treatments. At no time was there any purple or red tint, as with May King with phosphorus deficiency. By 16 October, treatment with solution E/P gave a slightly darker plant than treatments with A/P, B/NPK, C/P, and D/P, while F/P and G/P were slightly lighter in shade than these. But by 5 November, and during the remainder of the experiment, the colour was equivalent for all treatments except for the three cultures with G/P (no phosphorus), which were a shade or two lighter green than any others.

With solution E/P there was marginal chlorosis, and the leaves tended to curl under at the edges to give a wrinkled and ugly plant, which looked smaller than it really was; there was later some bleaching and russeting of the leaf margins. Treatments with F/P and G/P, on the other hand, resulted in non-wrinkled plants with bleached and russeted older leaves that subsequently died; these non-crinkled cultures, however, had not the abnormal "smoothness" characteristic of lack of nitrogen.

Linear dimensions in cm. at harvest were: A/P, $15 \times 13{\cdot}8$; B/NPK, $11{\cdot}7 \times 10{\cdot}5$; C/P, $12{\cdot}5 \times 11{\cdot}3$; D/P, $10 \times 9{\cdot}3$; E/P, 8×7; F/P, $3{\cdot}3 \times 2{\cdot}8$; and G/P (three cultures only), 4×4.

At harvest, eleven of A/P, seven of B/NPK, twelve of C/P, and three of D/P, showed definite signs of hearting. As with May King, a good supply of phosphorus tended to earlier maturity (Woodman, 1936, 1939 *a*).

When examined at harvest the roots were pale amber tap roots (bushier than for the corresponding nitrogen treatments) which were smaller in size and in quantity of attached fibre as the phosphorus decreased.

Potassium portion of the experiment. The cultures were the same green throughout the experiment for all treatments except with G/K (absence of potassium); these three had become slightly lighter than the others by 30 October, and remained so.

There was a tendency for deficiency in potash to lead to a non-wrinkled lettuce, but in no instance was this of the abnormally "smooth" type characteristic of lack of nitrogen.

With E/K, F/K, and G/K, marginal scorch and small patches of scorch were worse than with the preceding treatments. The plants were vigorous, however, and this scorch could not be considered very serious for their size.

The linear dimensions in cm. at harvest were: A/K, 12×11; B/NPK, $11{\cdot}7 \times 10{\cdot}5$;

C/K, 12×11; D/K, 11×10; E/K, 11·5×10·7; F/K, 8·8×8; and G/K (three cultures only), 9×6·7. As with May King, there was a singular lack of response to potash over a very wide range of concentration, A/K to E/K, and the size of lettuces with A/K was thus much less than those with the corresponding treatments for nitrogen, A/N, and phosphorus, A/P. Treatments with F/K and G/K apparently gave moderately large, vigorous lettuces: but reference to Table 3 shows that the differences in linear dimensions between treatments with G/K and, say, E/K, represented large differences in yield; in this connexion also, it is perhaps possible that the sodium present in the nitrate and phosphate supplied to the cultures might to some extent have replaced the missing potassium (Miller, 1931).

At harvest nine with A/K, seven each with B/NPK, C/K, D/K and E/K, three of F/K, and none of G/K, showed definite signs of hearting. It was thus only when the potassium was reduced to very small proportions, as in F/K, that maturity was delayed, a similar conclusion to that arrived at with May King (Woodman, 1936, 1939*c*, *d*).

The roots were similar to those obtained in the corresponding nitrogen and phosphorus treatments.

The yields, etc., with different treatments. The data are presented in Table 3.

TABLE 3. *Summaries of results for Cheshunt Early Giant lettuce*

Description of data		Treatment mean for A	B/NPK	C	D	E	F	General mean	S.E.	G
Tops, FW	SSS (N)	22·99	11·23	5·75	2·02	0·567	—	8·479	1·088	0·525
		A/N > B/NPK > C/N > D/N = E/N								
	(P)	17·40	—	12·52	6·99	3·41	0·369	—	—	0·573
		A/P > B/NPK = C/P > D/P > E/P = F/P								
	(K)	11·33	—	10·64	8·30	9·08	4·59	—	—	3·79
		A/K = B/NPK = C/K = D/K = E/K > F/K								
Roots, FW	SSS (N)	1·478	0·995	0·741	0·383	0·065	—	0·7595	0·1387	0·052
		A/N > B/NPK = C/N > E/N; B/NPK > D/N = E/N; C/N = D/N								
	(P)	2·185	—	0·902	0·573	0·304	0·074	—	—	0·078
		A/P > B/NPK = C/P > E/P = F/P; B/NPK > D/P > F/P; C/P = D/P; D/P = E/P								
	(K)	1·075	—	0·824	0·659	0·798	0·338	—	—	0·238
		A/K = B/NPK = C/K = E/K > F/K; A/K = B/NPK = C/K = D/K = E/K; D/K = F/K								
Whole plants, FW	SSS (N)	24·48	12·22	6·491	2·405	0·631	—	9·238	1·216	0·577
		A/N > B/NPK > C/N > D/N = E/N								
	(P)	19·58	—	13·42	7·561	3·801	0·443	—	—	0·651
		A/P > B/NPK = C/P > D/P > E/P = F/P								
	(K)	12·32	—	11·47	8·958	9·876	4·924	—	—	4·031
		A/K = B/NPK = C/K = D/K = E/K > F/K								
Tops, DW	SSS (N)	1·299	0·710	0·449	0·170	0·057	—	0·5559	0·0589	0·049
		A/N > B/NPK > C/N > D/N = E/N								
	(P)	1·048	—	0·823	0·479	0·270	0·047	—	—	0·062
		A/P > B/NPK = C/P > D/P > E/P > F/P								
	(K)	0·728	—	0·691	0·590	0·647	0·331	—	—	0·262
		A/K = B/NPK = C/K = D/K = E/K > F/K								
Roots, DW	SSS (N)	0·105	0·070	0·078	0·050	0·015	—	0·0707	0·0157	0·012
		A/N > D/N = E/N; A/N = B/NPK = C/N > E/N; B/NPK = C/N = D/N								
	(P)	0·234	—	0·091	0·054	0·022	0·006	—	—	0·004
		A/P > B/NPK = C/P = D/P > F/P; A/P = B/NPK = C/P > E/P = F/P; D/P = E/P								
	(K)	0·099	—	0·080	0·059	0·070	0·027	—	—	0·022
		A/K = C/K > F/K; A/K = B/NPK = C/K = D/K = E/K; B/NPK = D/K = E/K = F/K								

Table 3 (cont.)

Description of data		Treatment mean for A	B/NPK	C	D	E	F	General mean	S.E.	G
Whole plants, DW	SSS (N)	1·403	0·780	0·527	0·220	0·072	—	0·6263	0·0628	0·061
		A/N > B/NPK > C/N > D/N = E/N								
	(P)	1·281	—	0·914	0·534	0·292	0·053	—	—	0·066
		A/P > B/NPK = C/P > D/P > E/P > F/P								
	(K)	0·827	—	0·771	0·649	0·718	0·358	—	—	0·284
		A/K = B/NPK = C/K = D/K = E/K > F/K								
Top/root, FW	SSS (N)	15·52	12·44	8·59	5·89	9·46	—	11·19	0·8976	9·96
		A/N > B/NPK > C/N = E/N > D/N								
	(P)	10·16	—	14·89	12·15	11·43	4·52	—	—	7·34
		A/P = B/NPK = D/P = E/P > F/P; C/P > A/P = D/P = E/P; B/NPK = C/P								
	(K)	11·09	—	13·25	12·61	12·03	13·78	—	—	14·38
		A/K = B/NPK = C/K = D/K = E/K = F/K								
Top/root, DW	SSS (N)	12·29	10·70	6·74	3·93	3·88	—	9·09	0·9705	4·26
		A/N = B/NPK > C/N = D/N = E/N								
	(P)	6·42	—	10·11	9·75	12·29	8·54	—	—	15·33
		E/P > A/P = F/P; B/NPK = C/P = D/P = E/P > A/P; B/NPK = C/P = D/P = F/P								
	(K)	8·51	—	9·29	10·33	9·99	13·55	—	—	10·43
		F/K > A/K = C/K = D/K = E/K; B/NPK = F/K; A/K = B/NPK = C/K = D/K = E/K								
Tops, % moisture	SSS (N)	94·21	93·70	91·74	91·19	89·81	—	92·05	0·6468	90·41
		A/N = B/NPK > C/N = D/N = E/N								
	(P)	93·85	—	93·38	92·83	91·74	84·43	—	—	89·02
		A/P = B/NPK > E/P > F/P; C/P = D/P = E/P > F/P; A/P = B/NPK = C/P = D/P								
	(K)	93·17	—	93·25	92·81	92·39	92·22	—	—	92·84
		A/K = B/NPK = C/K = D/K = E/K = F/K								
Roots, % moisture	SSS (N)	93·05	92·90	89·30	86·03	73·86	—	89·62	0·7893	77·99
		A/N = B/NPK > C/N > D/N > E/N								
	(P)	89·49	—	89·85	90·75	92·61	92·24	—	—	94·25
		B/NPK = E/P = F/P > A/P = C/P; B/NPK = D/P = E/P = F/P; A/P = C/P = D/P								
	(K)	90·83	—	90·26	91·04	90·63	91·45	—	—	89·79
		A/K = B/NPK = D/K = E/K = F/K; B/NPK > C/K; A/K = C/K = D/K = E/K = F/K								
Whole plants, % moisture	SSS (N)	94·17	93·73	91·44	90·43	88·17	—	91·97	0·4624	89·29
		A/N = B/NPK > C/N = D/N > E/N								
	(P)	93·37	—	93·13	92·67	92·05	87·19	—	—	89·70
		A/P = B/NPK = C/P = D/P > F/P; B/NPK > E/P > F/P; A/P = C/P = D/P = E/P								
	(K)	92·94	—	92·99	92·67	92·25	92·34	—	—	92·63
		A/K = B/NPK = C/K = D/K = F/K; B/NPK > E/K; A/K = C/K = D/K = E/K = F/K								

As it would be inconvenient to give the results of all the treatments in one line of subheadings under the "Treatment mean for" heading, they are tabulated in sections on three different lines under the general subheadings A, B/NPK, C, D, E, F, and G; reference to the "Description of data" column for the distinguishing letter, N, P, or K, will indicate whether a treatment is, for example, A/N, A/P, or A/K.

A subtable contains the summaries of results for all randomized treatments, including the N, P, and K sections; there is thus only one "General mean" and one "S.E." for a subtable. It should be noted that G/N, G/P, and G/K, which were left outside the randomized arrangement, are naturally not included in the subtables or comparisons of significance, but are given as a separate column "G" which follows the "S.E." one.

The comparisons of significance of the differences between the separate treatment means (in all the eleven subtables z was significant at the 0·1 % level, SSS) were, however, split into three sections to suit the N, P, and K portions of the subtable: thus, while it is of practical interest to note the significance or otherwise of differences arising from progressive diminutions in the supply of, say, nitrogen, it serves little useful purpose in general to compare the results for a deficiency of nitrogen with that for phosphorus or potassium.

Nitrogen portion of the subtables. A progressive diminution in the amount of nitrogen supplied to the cultures resulted in progressive reductions in yield (as fresh or dry weights) of the tops, roots, and whole plants, till negligible yields were obtained with the least amount (E/N), and with none (G/N). The effect of doubling the amount of nitrogen in the control, as in solution A/N, was to double the fresh weight of the lettuce itself; this was far greater than the corresponding effect got by doubling the phosphorus, as in A/P, where a large significant increase was also obtained, or doubling the potassium, as in A/K, where there was no response. It thus appears that to obtain the best yield of fresh top of this crop a good supply of nitrogen is essential.

The roots were also greatly increased by this extra nitrogen with A/N; but reference to the corresponding result with A/P demonstrates that phosphorus appeared to be the more important factor for lettuce roots, a fact borne out by the May King experiment (Table 2), where reduction in the amount of phosphorus from solution C to D caused a great reduction in yield of roots ($C > D$), whereas a simultaneous reduction of nitrogen, as in E, caused no further loss in yield ($D = E$).

Reduction in the nitrogen supply led to large reductions in the top/root ratio for fresh, and especially for dried, lettuces, pointing again to the fact that nitrogen encouraged top growth of this crop relatively more than root growth. Diminution in the nitrogen supply also led to substantial reductions in the percentages of moisture present in the tops, roots, and whole lettuces.

Phosphorus portion of the subtables. Reduction in the supply of phosphorus led to reduced yields of fresh or dried tops, roots, or whole lettuces. The great increases in yields of tops and roots obtained by doubling the phosphorus present in the control solution B/NPK, as in A/P, have been noted, and it has also been observed that the increase with the tops was much less than that got by a similar increase of nitrogen (A/N), although for the root the increase was much greater. To confirm this, it will be noticed that the top/root ratios were significantly less for solution A/P (most phosphorus) than for solution C/P (for the fresh weights), or solutions B/NPK, C/P, D/P, and E/P (for dried lettuces); thus though the yield of top was increased with A/P, the yield of root was increased relatively much more.

The moisture contents of the tops and whole plants were in general decreased by reduction in the supply of phosphorus, while those of the roots were increased.

Potassium portion of the subtables. There was no response to potassium for the tops or whole plants as fresh or dried yields over the wide range of concentration A/K to E/K (44·88–2·81 p.p.m. of K). Only with F/K (0·56 p.p.m.) or G/K (potassium absent) were the yields severely and significantly reduced. The yields with G/K, though relatively small, tended to prove that small amounts of potassium were derived from the sand and/or pots. The response for the roots between E/K and A/K was also not great.

Top/root ratios for the fresh weights were not significantly altered in passing from solutions A/K to F/K inclusive, although the general trend of all the results showed a bias

in favour of an increased ratio with decrease of potassium. A somewhat similar result was obtained with the ratio for the dry weights. In general, also, the moisture contents of the tops (especially), roots, and whole plants were not influenced greatly by alteration in the supply of potassium.

Calcium experiment with May King lettuce

The lettuces were grown in glazed culture jars each containing about 46 lb. of sand. The arrangement was a randomized-block one of twelve replications of four treatments with solutions A/Ca, B/Ca, C/Ca, and D/Ca, forty-eight cultures in all; particulars of these solutions are given in the third part of Table 2. The seed was sown in the jars on 28 June 1937, and had germinated in every jar by 5 July. Thinning out to leave one lettuce per pot was done on 8 July; the harvest was on 13 August 1937. During the experiment, 8·5 l. of the particular solution concerned was given to each culture, usually in lots of 400 c.c. three times a week. The greenhouse was unheated throughout this experiment, and temperatures fluctuated from 50 to 80° F. The daily average numbers of hours of sunshine during the period were: 1st week, commencing 3 July, 3·6; 2nd week, 6·5; 3rd, 2·5; 4th, 2·0; 5th, 5·0; and 6th, ending on the day of harvest, 13 August, 5·5.

Summary of observations made on the cultures. In general, size was the only difference noticed between the treatments, all solutions tending to yield normal plants which were seen to have normal roots at harvest. The linear measurements in cm. at harvest were: A/Ca, 27·3 × 25·2; B/Ca, 26·8 × 24·3; C/Ca, 26·1 × 24·3; and D/Ca, 25·3 × 22·4. At harvest the numbers showing definite signs of hearting were: A/Ca, 7; B/Ca, 6; C/Ca, 3; D/Ca, 0, so that increase in the supply of available calcium tended to earlier maturity. Three other lettuces with A/Ca were sending up flower stems.

Table 4. *Summaries of results for calcium experiment with May King lettuce*

Description of data		Treatment mean for A/Ca	B/Ca	C/Ca	D/Ca	General mean	S.E.
Tops, FW	SSS	49·83	43·07	35·32	29·60	39·45	3·127
		A/Ca = B/Ca > D/Ca; A/Ca > C/Ca = D/Ca; B/Ca = C/Ca					
Roots, FW	NS	6·67	7·09	6·63	5·87	6·564	0·4650
Whole plants, FW	SSS	56·49	50·15	41·95	35·48	46·02	3·018
		A/Ca = B/Ca > D/Ca; A/Ca > C/Ca = D/Ca; B/Ca = C/Ca					
Tops, DW	SSS	2·03	1·87	1·70	1·40	1·754	0·1534
		A/Ca = B/Ca > D/Ca; A/Ca = B/Ca = C/Ca; C/Ca = D/Ca					
Roots, DW	NS	0·38	0·34	0·30	0·34	0·3406	0·03693
Whole plants, DW	S	2·41	2·21	2·00	1·73	2·095	0·1578
		A/Ca = B/Ca > D/Ca; A/Ca = B/Ca = C/Ca; C/Ca = D/Ca					
Top/root, FW	NS	8·38	6·49	5·59	5·57	6·509	0·8127
Top/root, DW	NS	5·83	5·85	5·93	4·75	5·590	0·6465
Tops, % moisture	SSS	95·90	95·66	95·25	95·12	95·48	0·1329
		A/Ca = B/Ca > C/Ca = D/Ca					
Roots, % moisture	S	94·23	95·05	95·50	94·23	94·75	0·3445
		C/Ca > A/Ca = D/Ca; A/Ca = B/Ca = D/Ca; B/Ca = C/Ca					
Whole plants, % moisture	S	95·72	95·60	95·29	94·98	95·40	0·1666
		A/Ca = B/Ca > D/Ca; A/Ca = B/Ca = C/Ca; C/Ca = D/Ca					

The yields, etc., with different treatments. The results are given in Table 4, where it will be noticed that z was not significant (NS) for the fresh and dry weights of the roots and the top/root ratios for fresh and dry weights, but was very strongly significant (SSS) for sub-

tables other than those for the moisture in the roots and whole plants, and the dry weights of the whole plants, where z was significant (S).

The fresh weights of the tops and whole plants were significantly reduced in exactly similar fashion by reductions in the calcium, as also were the corresponding dry weights. The fair size of the plants with solution D/Ca, 0·45 p.p.m. of calcium, showed how little of this element was necessary in an available form for growth.

The moisture contents of the tops and whole plants suffered small but significant reductions as the supply of calcium was reduced, but the small variations in the moisture contents of the roots, though significant, were more complex.

Magnesium experiment with May King lettuce

The cultures were contained in new plant pots which had been boiled for 20 min. in paraffin wax. Eighty cultures were arranged in the form of sixteen replications of a randomized block of five treatments with solutions A/Mg, B/Mg, C/Mg, D/Mg, and E/Mg, particulars of which are given in the fourth part of Table 1. The seed was sown in the pots on 30 June 1937, and had germinated in every pot by 8 July. Thinning out of the seedlings to leave one per pot was done on 8 July; the harvest was 23 August 1937. During the experiment, 5850 c.c. of the particular medium concerned were given to each pot, usually in lots of 250 c.c. three times a week. The temperatures in the unheated greenhouse were the same as for the calcium experiment alongside; the daily average hours of sunshine were also the same with the addition of an average of 6·3 hr. for the extra 10 days period (till 23 August) taken for this experiment.

Summary of observations made on the cultures. In general, solutions A/Mg, B/Mg, and C/Mg, yielded normal lettuces; but there was a definite and strong tendency, with the two treatments with least magnesium, D/Mg and E/Mg, to etiolation of the oldest leaves and formation of a pink tint, so that these leaves presented a characteristic "bleached pink" or whitish pink appearance. With solution E/Mg, also, there was a tendency to lighter green plants, and even to definite etiolation of the whole of some of them.

The linear measurements at harvest in cm. were: A/Mg, $28·3 \times 25·8$; B/Mg, $27·3 \times 25·4$; C/Mg, $27·6 \times 25·4$; D/Mg, $27·6 \times 24·9$; and E/Mg, $25·9 \times 21·7$. The following were the numbers of lettuces which showed definite signs of hearting at harvest; the figures in parentheses are those for additional lettuces which were then shooting up into flower stems: A/Mg, 12 (4); B/Mg, 11 (4); C/Mg, 14 (2); D/Mg, 13 (1); and E/Mg, 8 (1). Maturity was delayed to a slight extent by a definite lack of available magnesium as in E/Mg.

The yields, etc., with different treatments. The results are presented in Table 5, where it will be seen that z was very strongly significant (SSS) for every summary of results, except the moisture in the roots, where it was significant (S).

When the concentration of available magnesium supplied to the lettuces was lowered over the range represented by solutions A/Mg to E/Mg, the fresh and dry weights of the tops, roots, and whole plants, were reduced. The greatest relative reduction was suffered by the roots. This was borne out by the top/root ratios for fresh and dry weights; here it was found that the reductions of magnesium caused corresponding large increases in the ratios, that is, reduced the size of the roots relatively much more than the tops. It will be noted, from the yields with D/Mg and E/Mg, 0·25 and 0·13 p.p.m. of magnesium respectively, that very little available magnesium was needed to give fair growth.

Reduction in the amount of magnesium applied also caused small but significant increases in the moisture contents of the tops, roots, and whole plants.

TABLE 5. *Summaries of results for magnesium experiment with May King lettuce*

Description of data		Treatment mean for A/Mg	B/Mg	C/Mg	D/Mg	E/Mg	General mean	S.E.
Tops, FW	SSS	40·71	38·99	36·23	33·21	24·64	34·76	1·525
		A/Mg = B/Mg > D/Mg > E/Mg; A/Mg = B/Mg = C/Mg; C/Mg = D/Mg > E/Mg						
Roots, FW	SSS	12·98	11·77	11·20	7·51	3·50	9·392	0·6166
		A/Mg = B/Mg = C/Mg > D/Mg > E/Mg						
Whole plants, FW	SSS	53·69	50·75	47·43	40·72	28·14	44·15	1·956
		A/Mg > C/Mg > D/Mg > E/Mg; A/Mg = B/Mg > D/Mg; B/Mg = C/Mg						
Tops, DW	SSS	3·02	2·54	2·44	2·09	1·49	2·316	0·1120
		A/Mg > B/Mg = C/Mg > D/Mg > E/Mg						
Roots, DW	SSS	1·23	1·14	0·96	0·59	0·30	0·849	0·05602
		A/Mg = B/Mg > C/Mg > D/Mg > E/Mg						
Whole plants, DW	SSS	4·26	3·68	3·42	2·68	1·78	3·165	0·1570
		A/Mg > B/Mg = C/Mg > D/Mg > E/Mg						
Top/root, FW	SSS	3·19	3·40	3·41	5·23	7·93	4·633	0·3347
		E/Mg > D/Mg > A/Mg = B/Mg = C/Mg						
Top/root, DW	SSS	2·49	2·24	2·62	3·98	5·71	3·409	0·2394
		E/Mg > D/Mg > A/Mg = B/Mg = C/Mg						
Tops, % moisture	SSS	92·57	93·51	93·25	93·65	93·81	93·36	0·1443
		E/Mg > C/Mg > A/Mg; B/Mg = C/Mg = D/Mg > A/Mg; B/Mg = D/Mg = E/Mg > A/Mg						
Roots, % moisture	S	90·50	90·15	91·18	91·83	91·24	90·99	0·3417
		C/Mg = D/Mg = E/Mg > A/Mg = B/Mg						
Whole plants, % moisture	SSS	92·08	92·77	92·80	93·42	93·54	92·92	0·1443
		D/Mg = E/Mg > B/Mg = C/Mg > A/Mg						

SUMMARY

May King lettuce has been shown to respond well to nitrogen and phosphorus both as regards yield and earliness of maturity, but to make practically no response to potassium over a wide range of concentrations. Cheshunt Early Giant lettuce behaved similarly, but no development of purple flushes or tints took place with this non-tinted lettuce when there was a deficiency of phosphorus or nitrogen; a deficiency of nitrogen, however, resulted in a light green lettuce of a characteristic and abnormally regular and smooth appearance.

Lack of calcium with May King did not cause deficiency symptoms other than that of decreased yield. A deficiency symptom noted with magnesium was a tendency to an etiolated plant and to a bleached whitish pink appearance of the older leaves.

Yields and other data have been examined statistically.

I take this opportunity of expressing my best thanks to my assistant, Mr T. W. McKean, for his help during these experiments.

REFERENCES

MILLER, E. C. (1931). *Plant Physiology*, pp. 254, 282, 283. London and New York.

WISHART, J. & SANDERS, H. G. (1935). *Principles and Practice of Field Experimentation.* Empire Cotton Growing Corporation.

WOODMAN, R. M. (1936). Pure silica sand as a basis for phosphate deficiency tests on lettuce. *Sands, Clays & Min.* **3**, 22–7.

—— (1939 *a*). Studies in the nutrition of vegetables. Phosphate deficiency and yield tests on sand cultures of May King lettuce. *J. agric. Sci.* **29**, 229–49.

—— (1939 *b*). Studies in the nutrition of vegetables. The effects of variation in the nitrogen supply on sand cultures of May King lettuce. *Ann. Bot., Lond.*, **3** (N.S.), 649–56.

—— (1939 *c*). Effects of variation in the supply of potash to lettuces grown under glass. *J. Pomol.* **17**, 167–80.

—— (1939 *d*). Studies in the nutrition of vegetables: the effects of varying the potash supply on sand cultures of lettuce. *Soil Sci.* **48**, 101–8.

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THE ECOLOGY OF THE LARGER FUNGI

IV. THE SEASONAL FREQUENCY OF GRASSLAND FUNGI WITH SPECIAL REFERENCE TO THE INFLUENCE OF ENVIRONMENTAL FACTORS

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(With 5 Text-figures)

1. Introduction

This paper continues previous work (Wilkins & Patrick, 1939), and the remarks on the conditions under which that work was done apply here. It is a more intensive study of the seasonal variation of the fungi of grasslands with particular reference to the environmental factors, in addition to soil type, which might influence the appearance of the fungi in time and abundance. It was not possible to include all the twenty stations previously examined and observations were restricted to three station, one on each of three types of soil. The open nature of grasslands makes them particularly favourable for work on this aspect of fungus ecology, as complications which might arise from the presence of a changing canopy of vegetation are avoided. The object of the investigation was to determine the seasonal variation of the fungus population and to correlate this variation with such factors of the environment as appeared to be contributory, that is to find out how and why the numbers of the grassland fungi varied throughout the year.

2. Experimental methods and data

Three grassland stations on chalk, clay and sand, respectively, were visited at approximately fortnightly intervals during the period 1 January 1937 to 1 January 1938. The intervals between successive visits were from 12 to 16 days with, in one case, an interval of a month. To ensure that environmental conditions might be as similar as possible the stations were as near together as the different soil types permitted, and were Crowell Hill (chalk), Horspath Meadows (clay), and Nuneham Park (sand). They will be referred to subsequently as "chalk", "clay" and "sand". These were near Oxford and within 13 miles of each other. Notes on the soil character and floristic composition of these areas are given by Wilkins & Patrick (1939). On each station an area 100 yd. square was marked out. These areas were selected as having given a reasonably high number of fungal species in the examinations of the previous season, as, for our present purpose, random selection has no particular significance.

At each visit records were made of temperature, rainfall, and water content and *p*H of the soil. The number of fungal species and individuals was also calculated.

(*a*) *Temperature*

Temperature was recorded by maximum and minimum thermometers placed at ground-level and protected by wire cages. Only fortnightly readings could be obtained, but a comparison between these and the readings from similarly placed thermometers at Nuneham Park (made available by the kindness of the head gardener) showed that variation was sufficiently small to be ignored, and the daily records from Nuneham can be taken as representative of all areas. Temperatures are given in Figs. 2 and 3.

(*b*) *Rainfall*

Rain gauges set up on the three areas were protected by wire frames, but experience showed that these frames made no appreciable difference to the records. On all three stations the rainfall figures were very similar, though chalk had a slightly higher rainfall than either of the other two stations. The total annual rainfalls were chalk 29·67 in., clay 24·04 in., sand 23·44 in.

From the standpoint of fungal development it was considered that the water content of the soil had more direct effect than rainfall.

(*c*) *Water content of the soil*

As a general rule, six soil samples were taken from each station at each visit. They were mixed samples taken to a depth of 2 in. at more or less regular intervals over the stations. The water content was estimated in terms of loss of weight after drying at 90° C. Owing to changes in organic matter, etc., it was difficult to obtain a constant weight, so 24 hr. was taken as the standard time for drying.

The amount of water present in air-dried soil was also calculated; soil which had been air dried for 14 days was oven dried for 24 hr. and loss of weight expressed as a percentage of weight of air-dry soil (see Table 1). Comparison between air-dried soil and oven-dried soil was not made.

TABLE 1. *Estimation of water content of air-dry soil*

Station	Total wt. of air-dry soil, in g.	Wt. after oven drying, in g.	% water content
Chalk	718·4	695·8	7·8
Clay	747·8	732·8	5·7
Sand	650·5	639·1	5·5

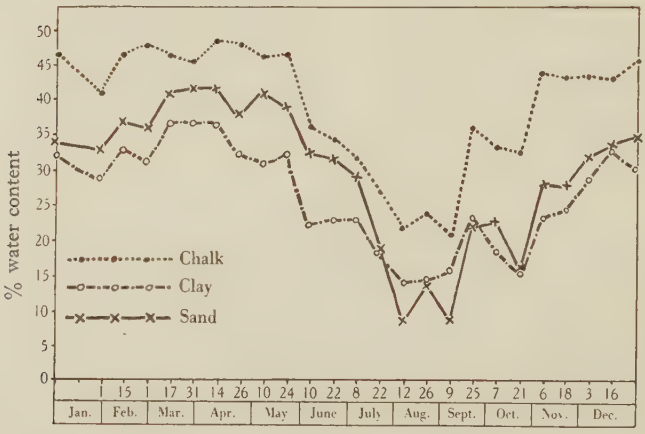

Fig. 1. Comparative seasonal variation in water content of the chalk, clay and sand soils.

In the case of the soil samples, the water loss was expressed as a percentage of the fresh weight. There is some difference in the average water content of the three soils over the year: chalk 39·4 %, clay 26·3 %, sand 29·7 %. The interesting feature is the variation in soil-water content throughout the year (see Fig. 1).

Chalk had the highest water content, the retention being due to the higher percentage of organic matter, sand was next and the clay lowest, except during the period 22 July–25 September when sand was the lowest of the three. In general the seasonal variation was very similar in all three cases. The water content was high from January to the end of May, followed by a gradual decrease which reached its maximum from about mid-August to mid-September. After that it gradually rose again until January.

(*d*) *Hydrogen-ion concentration*

The *p*H was calculated by taking six determinations on each station at each visit, by the B.D.H. Capillator method (see Table 2). The object was to ascertain if there was (1) any seasonal variation in *p*H and (2) any significant difference between the *p*H values of the respective soils.

TABLE 2. *p*H *determinations on the three stations*

Chalk		Clay		Sand	
Range	Mean	Range	Mean	Range	Mean
6·8–7·1	7·0	5·8–6·3	6·1	4·6–5·0	4·9

Though distribution and numbers of fungi may be affected by the different *p*H of the different stations the seasonal variation of *p*H on each station was very small and, from a knowledge of the *p*H toleration of fungi in culture, the above seasonal variation is not considered to be sufficient to influence the seasonal variation of the fungi. It is interesting to compare this *p*H variation with that found by Baker & Clapham (1939) in woodland soils, where a distinct seasonal variation in hydrogen-ion concentration occurred.

(*e*) *The fungus flora in general*

At each visit to each station the fungal sporophores were collected, counted and destroyed. The method of counting was by the "zigzag transect", and the average time taken was 2 hr. From the records it was possible to make lists showing the seasonal appearance and frequency of the species on each type of soil. These details are given in Table 3, which shows the

TABLE 3. *List showing distribution of species and frequency of individuals on the three soil types*

Chalk		Clay		Sand	
2 Jan.	No.	1 Jan.	No.	3 Jan.	No.
Clitocybe cyathiformis	6	*Clavaria corniculata*	4	*Clavaria fusiformis*	3
C. fragrans	2	*C. flaccida*	2	*Clitocybe fragrans*	4
Lepiota granulosa	3	*Naucoria melinoides*	2	*Hygrophorus coccineus*	2
Lycoperdon depressum	2	*Tricholoma personatum*	2	*H. pratensis*	1
Panaeolus campanulatus	4			*H. puniceus*	1
Stropharia semiglobata	1			*Lycoperdon nigrescens*	1
				Naucoria melinoides	8
				Panaeolus campanulatus	5
				P. sphinctrinus	5
				Peziza aurantia	4
				Psilocybe semilanceata	1
				Stropharia semiglobata	4
2 Feb.		1 Feb.		3 Feb.	
Lycoperdon depressum	5	*Lycoperdon depressum*	1	*Clavaria fusiformis*	2
Naucoria melinoides	3	*Naucoria melinoides*	1	*Hygrophorus pratensis*	1
Panaeolus campanulatus	12	*Panaeolus campanulatus*	5	*H. psittacinus*	5
		Tubaria inquilina	6	*Lycoperdon depressum*	12
				L. nigrescens	2
				L. pusillum	8
				Naucoria melinoides	3
				Panaeolus campanulatus	2
				P. sphinctrinus	1
				Stropharia semiglobata	2

Table 3 (cont.)

Chalk		Clay		Sand	
16 Feb.	No.	15 Feb.	No.	17 Feb.	No.
Lycoperdon depressum	5	*Clavaria corniculata*	1	*Galera hypnorum*	1
L. pusillum	8	*Panaeolus campanulatus*	1	*Lycoperdon depressum*	3
Panaeolus campanulatus	15	*Tubaria inquilina*	15	*Naucoria melinoides*	6
Tubaria inquilina	11			*Nolanea proletaria*	1
				Panaeolus campanulatus	26
				P. sphinctrinus	6
				Stropharia aeruginosa	1
				S. semiglobata	1
3 Mar.		2 Mar.		1 Mar.	
Panaeolus campanulatus	10			*Galera hypnorum*	1
Tubaria inquilina	59			*Lycoperdon depressum*	3
				Naucoria melinoides	4
				Panaeolus campanulatus	2
				Tubaria inquilina	12
15 Mar.		18 Mar.		17 Mar.	
Panaeolus campanulatus	5	*Tubaria inquilina*	2	*Lycoperdon depressum*	2
Tubaria inquilina	11			*Naucoria melinoides*	2
				Tubaria inquilina	6
1 Apr.		1 Apr.		31 Mar.	
Panaeolus campanulatus	9			*Lycoperdon depressum*	2
				Panaeolus campanulatus	4
				Stropharia semiglobata	2
15 Apr.		14 Apr.		14 Apr.	
Panaeolus campanulatus	108	*Psilocybe foenisecii*	2	*Hygrophorus psittacinus*	1
Psilocybe foenisecii	22			*Naucoria melinoides*	26
				Nolanea proletaria	11
				Panaeolus campanulatus	24
				P. sphinctrinus	4
				Psilocybe foenisecii	36
				P. semilanceata	2
				Stropharia semiglobata	1
27 Apr.		27 Apr.		26 Apr.	
Bolbitius intellinus	2	*Tricholoma gambosum*	3	*Psilocybe foenisecii*	12
Panaeolus campanulatus	12				
Psilocybe foenisecii	15				
Tricholoma gambosum	59				
11 May		10 May		10 May	
Bolbitius intellinus	1	*Marasmius oreades*	24	*Coprinus plicatilis*	4
Galera hypnorum	1	*Tricholoma gambosum*	51	*Marasmius oreades*	6
Naucoria semiorbicularis	2			*Naucoria semiorbicularis*	17
Psilocybe ericaea	3			*Nolanea proletaria*	8
Tricholoma gambosum	37			*Psilocybe foenisecii*	1
Tubaria inquilina	11			*Stropharia semiglobata*	1
26 May		25 May		24 May	
Bolbitius intellinus	7	*Marasmius oreades*	102	*Coprinus plicatilis*	43
Galera tenera	1	*Tricholoma gambosum*	226	*Galera hypnorum*	5
Inocybe lacera	5			*G. tenera*	13
Naucoria semiorbicularis	85			*Naucoria semiorbicularis*	168
Panaeolus campanulatus	7			*Nolanea proletaria*	15
Stropharia merdaria	1			*Panaeolus sphinctrinus*	28
Tricholoma gambosum	18			*Psilocybe foenisecii*	8
T. melaleucum	2				
Tubaria inquilina	30				

Table 3 (cont.)

Chalk		Clay		Sand	
11 June	No.	10 June	No.	10 June	No.
		Marasmius oreades	1	*Galera tenera*	1
		Psaliota campestris	1	*Naucoria semiorbicularis*	2
		Tricholoma gambosum	2	*Panaeolus sphinctrinus*	7
22 June		22 June		23 June	
Galera tenera	1	*Marasmius oreades*	110	*Coprinus plicatilis*	6
Naucoria semiorbicularis	98	*Psaliota campestris*	1	*Galera tenera*	1
		Psilocybe foenisecii	18	*Marasmius oreades*	1
		Tricholoma gambosum	1	*Naucoria semiorbicularis*	190
				Panaeolus campanulatus	2
				P. sphinctrinus	16
				Psilocybe foenisecii	42
9 July		9 July		8 July	
Naucoria semiorbicularis	109	*Marasmius oreades*	23	*Clitocybe infundibuliformis*	17
				Naucoria semiorbicularis	16
23 July		23 July		22 July	
Clitocybe infundibuliformis	3	*Marasmius oreades*	25	*Clitocybe infundibuliformis*	50
Naucoria semiorbicularis	104	*Psaliota campestris*	1	*Naucoria semiorbicularis*	19
Psilocybe foenisecii	8	*Psilocybe foenisecii*	6		
13 Aug.		12 Aug.		12 Aug.	
—		—		—	
27 Aug.		26 Aug.		26 Aug.	
Naucoria semiorbicularis	8				
10 Sept.		9 Sept.		9 Sept.	
Clitocybe infundibuliformis	1			*Naucoria semiorbicularis*	11
C. rivulosa	3				
Galera hypnorum	2				
Lycoperdon depressum	1				
Marasmius dryophilus	12				
Naucoria semiorbicularis	44				
26 Sept.		26 Sept.		25 Sept.	
Clitocybe infundibuliformis	42	*Clitopilus cancrinus*	2	*Clitocybe aurantiaca v. albida*	3
C. rivulosa	53	*Coprinus niveus*	12	*C. infundibuliformis*	14
Clitopilus prunulus	3	*C. plicatilis*	3	*Coprinus plicatilis*	2
Coprinus plicatilis	2	*Marasmius oreades*	7	*Galera hypnorum*	3
Galera hypnorum	172	*Psaliota campestris*	8	*G. tenera*	15
G. tenera	9	*Stropharia semiglobata*	31	*Lycoperdon depressum*	4
Lepiota clypeolaria	14			*L. pusillum*	1
L. gracilenta	2			*Naucoria semiorbicularis*	15
Lycoperdon depressum	22			*Omphalia fibula*	13
L. perlatum	14			*Panaeolus sphinctrinus*	3
L. pusillum	5			*Stropharia semiglobata*	71
Marasmius dryophilus	19				
Mycena avenacea	3				
M. epipterygia	44				
M. pelliculosa	2				
M. pura	20				
Naucoria semiorbicularis	11				
Nolanea proletaria	1				
Panaeolus papilionaceus	1				
Psaliota arvensis	1				
P. dulcidula	12				
Stropharia semiglobata	2				

Table 3 (cont.)

Chalk		Clay		Sand	
8 Oct.	No.	8 Oct.	No.	7 Oct.	No.
Clitocybe infundibuliformis	11	*Anellaria separata*	1	*Clitocybe infundibuliformis*	16
C. rivulosa	155	*Clitocybe rivulosa*	53	*C. rivulosa*	20
Clitopilus prunulus	1	*Clitopilus cancrinus*	46	*Galera hypnorum*	7
Cortinarius anomalus	3	*Coprinus niveus*	1	*Lepiota gracilenta*	2
Galera hypnorum	202	*Leptonia lampropus*	3	*L. permixta*	3
G. tenera	2	*Lycoperdon depressum*	3	*L. procera*	7
Hebeloma crustuliniforme v. minus	2	*Marasmius oreades*	11	*Marasmius erythropus*	25
Inocybe rimosa	1	*Nolanea proletaria*	5	*M. oreades*	3
Lepiota clypeolaria	13	*Psaliota campestris*	10	*Naucoria melinoides*	17
L. cristata	1	*Psilocybe foenisecii*	3	*Omphalia fibula*	4
L. gracilenta	10	*P. semilanceata*	1	*Psilocybe semilanceata*	116
Lycoperdon depressum	3	*Stropharia semiglobata*	2	*Stropharia semiglobata*	11
L. perlatum	3	*Tubaria furfuracea*	1		
L. umbrinum	1				
Marasmius dryophilus	3				
M. erythropus	1				
Mycena epipterygia	343				
M. pura	29				
Naucoria cerodes	9				
N. semiorbicularis	1				
N. striaepes	1				
Omphalia fibula	1				
Stropharia aeruginosa	4				
S. inuncta	6				
Tricholoma terreum	12				
22 Oct.		22 Oct.		21 Oct.	
Clitocybe infundibuliformis	3	*Clitocybe rivulosa*	4	*Clitocybe rivulosa*	1
C. rivulosa	46	*Coprinus niveus*	3	*Lycoperdon nigrescens*	4
Cortinarius anomalus	3	*Hygrophorus coccineus*	3	*Marasmius erythropus*	4
Galera hypnorum	42	*Marasmius oreades*	1	*Psilocybe semilanceata*	3
Hygrophorus ovinus	1	*Psaliota campestris*	33	*Stropharia semiglobata*	1
Lepiota clypeolaria	3	*Tricholoma personatum*	3		
Lycoperdon depressum	2				
L. perlatum	1				
L. umbrinum	1				
Mycena epipterygia	93				
M. pura	3				
Psaliota dulcidula	3				
Stropharia aeruginosa	2				
S. inuncta	6				
S. semiglobata	1				
Tricholoma melaleucum	7				
T. terreum	4				
7 Nov.		8 Nov.		6 Nov.	
Clavaria dissipabilis	1	*Anellaria separata*	7	*Bolbitius intellinus*	1
Clitocybe fragrans	2	*Bovista plumbea*	1	*Clavaria fusiformis*	2
C. infundibuliformis	1	*Clitocybe rivulosa*	10	*C. tenuipes*	11
C. rivulosa	66	*Clitopilus cancrinus*	2	*Clitocybe fragrans*	10
Coprinus plicatilis	6	*Coprinus niveus*	94	*C. infundibuliformis*	6
Cortinarius anomalus	1	*C. plicatilis*	26	*C. rivulosa*	2
Entoloma sericeum	1	*Entoloma sericeum*	4	*Entoloma sericeum*	12
Galera hypnorum	135	*Galera tenera*	7	*Galera hypnorum*	97
G. tenera	41	*Hygrophorus chlorophanus*	2	*G. tenera*	43
Hygrophorus Colemannianus	3	*H. coccineus*	2	*Hygrophorus ovinus*	2
H. virgineus	8	*H. psittacinus*	3	*H. pratensis*	1
H. virgineus v. roseipes	4	*H. virgineus*	16	*H. virgineus*	10
Lepiota gracilenta	6	*H. virgineus v. roseipes*	2	*H. virgineus v. roseipes*	10

Table 3 (cont.)

Chalk		Clay		Sand	
7 Nov.	No.	8 Nov.	No.	6 Nov.	No.
Lepiota granulosa	4	*Lycoperdon depressum*	1	*Lepiota gracilenta*	1
Lycoperdon depressum	3	*Mycena ammoniaca*	19	*L. granulosa*	1
L. perlatum	10	*M. avenacea*	8	*Lycoperdon depressum*	2
Mycena avenacea	1	*M. flavoalba*	11	*L. nigrescens*	10
M. epipterygia	563	*M. metata*	15	*L. perlatum*	4
M. filopes	6	*Naucoria melinoides*	6	*L. pusillum*	6
M. metata	90	*Nolanea proletaria*	6	*Marasmius erythropus*	19
M. pelliculosa	7	*Panaeolus papilionaceus*	3	*Mycena ammoniaca*	15
Naucoria badipes	3	*P. sphinctrinus*	3	*M. avenacea*	25
N. melinoides	25	*Psaliota campestris*	30	*M. metata*	103
Omphalia fibula	8	*Psilocybe semilanceata*	16	*M. pelliculosa*	3
Panaeolus campanulatus	1	*Stropharia aeruginosa*	1	*Naucoria melinoides*	18
Psaliota campestris	2	*S. semiglobata*	32	*Omphalia fibula*	212
Stropharia aeruginosa	3	*Tricholoma personatum*	10	*Panaeolus campanulatus*	49
S. inuncta	3			*P. papilionaceus*	11
S. semiglobata	3			*P. sphinctrinus*	1
Tricholoma carneum	9			*Psaliota comtula*	1
T. melaleucum	41			*Psilocybe semilanceata*	308
T. sordidum	1			*Stropharia aeruginosa*	9
T. terreum	1			*S. albocyanea*	1
T. terreum v. strosquamosum	9			*S. semiglobata*	26
19 Nov.		19 Nov.		18 Nov.	
Clitocybe rivulosa	2	*Clavaria corniculata*	3	*Galera hypnorum*	2
Hygrophorus virgineus	1	*Clitocybe rivulosa*	2	*Lycoperdon nigrescens*	15
Lycoperdon perlatum	4	*Entoloma jubatum*	3	*Naucoria melinoides*	25
Mycena epipterygia	22	*Psaliota campestris*	6	*Omphalia fibula*	13
Naucoria badipes	4	*Stropharia semiglobata*	1	*Psilocybe semilanceata*	8
N. melinoides	3	*Tricholoma personatum*	21	*Stropharia semiglobata*	2
Psaliota campestris	2				
Stropharia aeruginosa	1				
Tricholoma carneum	3				
T. melaleucum	9				
T. sordidum	4				
Tubaria inquilina	9				
4 Dec.		4 Dec.		3 Dec.	
Clitocybe rivulosa	5	*Clitocybe rivulosa*	3	*Clitocybe fragrans*	9
Galera tenera	9	*Hygrophorus virgineus*	9	*Hygrophorus virgineus*	5
Naucoria melinoides	5	*H. virgineus v. roseipes*	7	*Lycoperdon nigrescens*	11
Tricholoma melaleucum	2	*Panaeolus papilionaceus*	1	*Mycena metata*	3
Tubaria inquilina	11	*Stropharia semiglobata*	11	*Naucoria melinoides*	35
		Tricholoma personatum	14	*Omphalia fibula*	26
				Psilocybe semilanceata	10
17 Dec.		17 Dec.		16 Dec.	
Lepiota granulosa	1	*Hygrophorus virgineus*	2	*Lepiota granulosa*	1
Naucoria melinoides	6	*Tricholoma personatum*	8	*Stropharia semiglobata*	4
Tricholoma carneum	1				
Tubaria inquilina	26				
2 Jan.		2 Jan.		1 Jan.	
Clitocybe rivulosa	2	*Tricholoma personatum*	2	*Omphalia fibula*	1
Tubaria inquilina	7				

fungus population on each station at each visit, both in species and in numbers of individuals. The total number of individuals was highest on the chalk. In the previous paper, (Wilkins & Patrick, 1939) where twenty stations were considered, the greatest fungus population was on sand, and the high frequency of individuals on chalk in this case must be considered as fortuitous. The total number of species and individuals found on each station at each visit is summarized in Table 4.

TABLE 4. *Frequency of species and individuals on the three soil types*

	Chalk		Clay		Sand	
1937	Spp.	Indiv.	Spp.	Indiv.	Spp.	Indiv.
1 Jan.	6	18	4	10	12	39
1 Feb.	3	20	4	13	10	38
15 ,,	4	39	3	17	8	45
1 Mar.	2	69	—	—	5	22
17 ,,	2	16	1	2	3	10
31 ,,	1	9	—	—	3	8
14 Apr.	2	130	1	2	8	105
26 ,,	4	88	1	3	1	12
10 May	6	55	2	75	6	37
24 ,,	9	156	2	328	7	280
10 June	—	—	3	4	3	10
22 ,,	2	99	4	130	7	258
8 July	1	109	1	23	2	33
22 ,,	3	115	3	32	2	69
12 Aug.	—	—	—	—	—	—
26 ,,	1	8	—	—	—	—
9 Sept.	6	63	—	—	1	11
25 ,,	22	454	6	63	11	144
7 Oct.	25	818	13	140	12	231
21 ,,	17	221	6	47	5	13
6 Nov.	34	1068	27	337	34	1032
18 ,,	12	64	6	36	6	65
3 Dec.	5	32	6	45	7	99
16 ,,	4	34	2	10	2	5
1 Jan.	2	9	1	2	1	1
Total		3694		1319		2567

Table 4 shows that the seasonal variation of the fungus population was very similar on all three stations. This applies both to species and to individuals, but there is some difference in that in the less favourable periods the number of species was always relatively high and the number of individuals relatively low. This has been noted before as characteristic of the beginning or end of a fungus season (Wilkins *et al.* 1938). The environmental factors are assumed to show approximately equal variation on all three stations so that whether the soil is chalk, clay or sand the seasonal variation in frequency of individuals is influenced by environment. There was, however, some difference of reaction to environment in relation to the station. Of the factors of the environment considered, it has already been shown that *p*H of the soil has no seasonal influence; rainfall can more adequately be expressed in terms of soil-water content, so that the only two significant factors seem to be temperature and soil-water content. The effect of these two factors on relative frequency of sporophore numbers will now be considered.

3. Relation between environmental factors and fungus numbers

As an example for analysis the state of affairs which obtained on the sand station (Nuneham Park) has been taken. Fig. 2 shows the mean weekly maximum and minimum temperatures, the water content of the soil and the number of sporophores recorded at each visit.

From Fig. 2 the following facts may be deduced:

(1) From 1 January to 31 March the fungus flora was scanty, due, most probably, to the low minimum temperature as water content was extremely high.

(2) A rise in the minimum temperature from 31 March to 14 April was accompanied by an increase in the number of fungi, and it would appear that the fall in the minimum temperature from 14 to 26 April was responsible for the decrease in the fungal numbers recorded on the latter date.

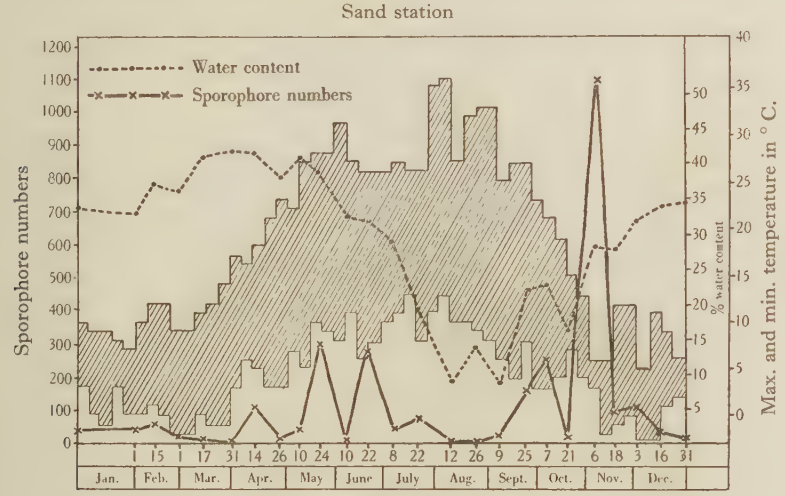

Fig. 2. Variation in fungus numbers in relation to temperature and water content.

(3) After 26 April there was a gradual general rise in minimum temperature which was accompanied by a corresponding increase in fungal numbers from that date to 22 June, with the exception of one sharp drop recorded on 10 June. This drop cannot be accounted for by any fall in minimum temperature (there was no sharp drop on any one day which would not appear in a weekly average) and water content was still too high to operate adversely. It seems, therefore, that this decrease in fungal numbers can be explained only by the high maximum temperature (over 30° C.) which immediately preceded that date.

(4) The period from 22 June to 9 September was characterized by a decrease in the fungus numbers apparently consequent upon a gradual falling water content. The period from 12 August to 9 September was especially poor, as at this stage the water content reached its lowest level and the maximum temperature was at its highest.

(5) From 9 September to 6 November there was, in general, a marked increase in fungus numbers which seems to be a response to the rise of the water content from about 10 % to about 25–30 %. It seems, also, that the sharp drop in numbers recorded on 21 October was

due to the fall in the water content at that time, this being specially effective in view of the relatively high maximum temperature obtaining then.

(6) From 6 November, which was the peak of the fungus season in this year, there was a spectacular drop in numbers of fungi by 18 November, and from then until the spring of the next year there was no further rise. This is a repetition of the state of affairs found in the early part of the year when the favourable influence of a high water content was negatived by a low minimum temperature.

A comparison between the fluctuation of fungi in response to environment on the chalk station (Fig. 3) with that on the sand station, shows that while the deductions are essentially the same there is some difference in detail.

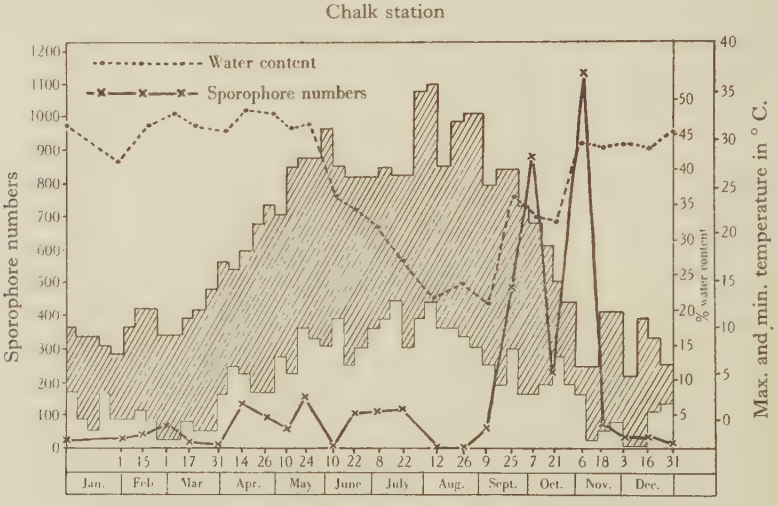

Fig. 3. Variation in fungus numbers in relation to temperature and water content.

(1) On the chalk station there was a consistently higher water content; it never dropped below 20 %.

(2) In the early months, January–10 May, the course of events is similar to that found on sand; there was the same rise on 14 April and a fall to 10 May.

(3) Though there was a rise in sporophore numbers on 24 May it was not so marked as on the sand station. Similarly, though there was the same decrease on 16 June due to the same reason as before, there was again a much less emphatic rise on 22 June. It seems as if the response to rise in minimum temperature was less vigorous.

(4) From 22 June to 22 July there was no decrease in fungus numbers. This was probably due to high water content of the chalk soil. On the other hand, the gradually falling water content probably prevented an actual rise, which minimum temperature would have allowed.

(5) The presence of few sporophores from 12 August to 9 September corresponds with the sand station, but the rise from 9 September to 7 October was much greater than the rise on sand at that time. This rise is the most striking difference between the two sets of results. It seemed to be conditioned by the rise in water content of the chalk soil, which increased to

35 % as compared with the increase to 20 % on the sand. The minimum temperature was lower than it had been previously, but the mean temperature was sufficiently high to allow the increase in water content to operate favourably.

(6) As in the case of sand, the decrease in water content recorded on 21 October was accompanied by a fall in sporophore numbers, though not to the same extent, and the subsequent events on chalk were as for sand.

In the case of the clay station the same type of conclusion can be drawn. Fig. 4 shows the condition on this station.

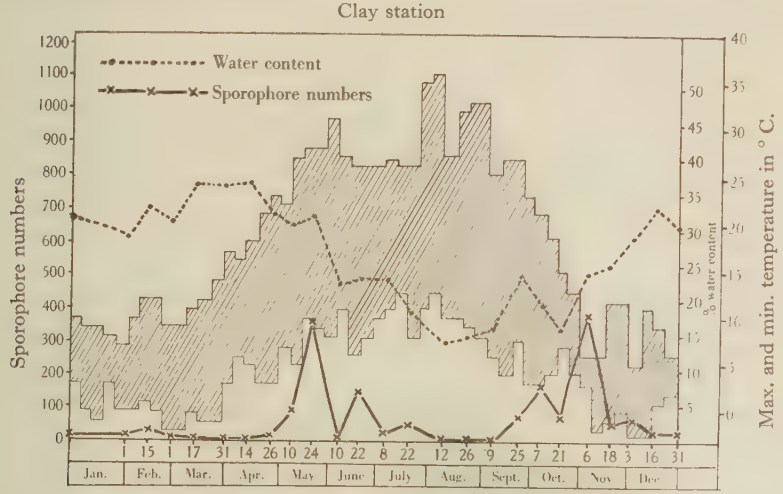

Fig. 4. Variation in fungus numbers in relation to temperature and water content.

(1) This station has a relatively low water content, and on the whole few sporophores were produced.

(2) In general, the state of affairs on clay follows the lines as indicated for the sand station, with the exception that the autumnal rise in sporophore numbers was comparatively small. There were, in fact, no more sporophores in the autumn season than in the summer season.

From the above analyses it would seem that the number of fungi produced is in direct relationship with the temperature and water content and that production of fungus sporophores, to any considerable extent, was conditioned by:

(1) A soil water content above a minimal value;

(2) A maximum temperature which is below a certain value; and

(3) A minimum temperature which is above a certain value.

It is reasonable to suggest that the well-known summer and autumn fungus seasons are due to the fact that only at these two periods of the year does a synchronization of favourable factors make sporophore production possible. The degree to which these environmental factors are favourable is, most probably, the determining influence on the variation in fungus numbers in different season. The term "favourable" is here used in the sense of conditions which are accompanied or followed by a rise in sporophore numbers; that sporophores may be produced as a result of conditions which are unfavourable to the growth of the mycelium is not overlooked.

The effect of these environmental factors on grassland fungi in general is shown in Fig. 5 where the total number of sporophores found on all three stations is plotted against average weekly temperature, and the average water content determined at fortnightly intervals. In this case the curves have been smoothed to eliminate sudden fluctuation in water content or sporophore numbers, and to anticipate what might happen in any average season.

The results confirm the more detailed analyses of the three individually located stations in that a low minimum temperature is the inhibiting factor in the winter months, while low

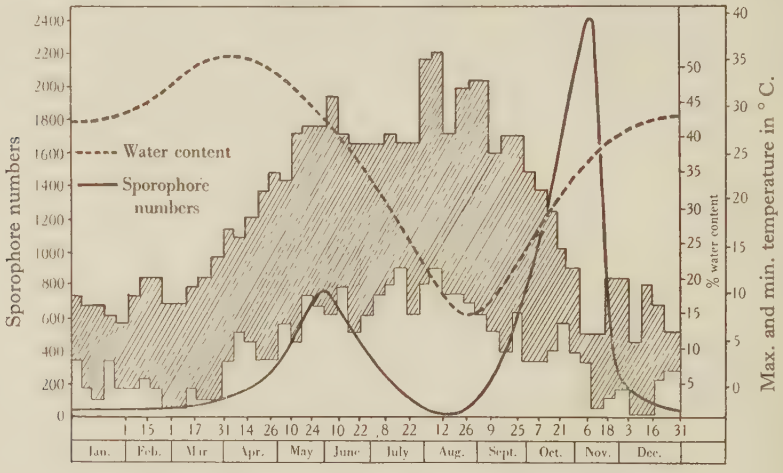

Fig. 5. Average seasonal variation in fungus numbers in relation to temperature and water content on all three stations.

TABLE 5. *Maximum sporophore production in relation to temperature and water content*

		Temp. ° C. Max.	Temp. ° C. Min.	Water content
Spring:	24 May	20	7	39·2
Autumn:	25 Sept.	26	5	28·0
	7 Oct.	26	7	24·9
	6 Nov.	17	6	31·7

water content and high maximum temperature are the limiting factors in the summer months. The environmental factors are only really favourable in summer (May–June) and in autumn (September–November). It is suggested that, in general, the summer and autumn fungus seasons are conditioned by the above mentioned factors. The operative range of these factors cannot be ascertained definitely on such slender evidence, but it is interesting to note the conditions which obtained at the times of maximum sporophore production (see Table 5).

It might, therefore, tentatively be suggested that the minimum temperature should be above 5° C., the maximum temperature below 30° C. and the water content above 20 %.

4. SEASONAL DISTRIBUTION OF GRASSLAND FUNGI

It seems evident from the foregoing records and graphs that fungal species can be grouped into three classes, according to the time of year, when they appear as shown below. The majority of species have a short fruiting season, but certain species seem to appear

sporadically during most of the year. Taking the species collectively the three classes are:

(1) A relatively large number of species that are found in maximum abundance about September–November and which constitute the autumn season.

(2) A comparatively small number of species that appear about May–July forming the summer season.

(3) A few species that occur in the remaining colder months of the year from December to April.

These fungus seasons are constituted, not only by the number of species which appear but also by the frequency of the individuals in each species.

(*a*) *Seasonal distribution of individual species*

The seasonal distribution of species with their individual frequency is given in an alphabetical list in Table 6.

By means of the frequency figures in Table 6 it is possible to determine when any species is at the maximum abundance, and from this can be deduced those species which are characteristic of the previously mentioned seasons.

In this particular instance the autumn season can be said to last from 9 September to 3 December, and Table 7 is a list of the species, with individual frequency, which are found then: species with a frequency below 10 have been excluded.

Similarly the summer season can be said to last from 26 April to 22 July, and Table 8 is a list of those species found during that time which had a frequency above 10.

In Tables 7 and 8 the number of species found in the autumn was 45 while in the summer only 12 species were found. In general, those species found in the summer were the same as those found in the autumn, the only exceptions being:

Bolbitius intellinus	*Tricholoma gambosum*
Psilocybe foenisecii	*Tubaria inquilina*

Of those species which are common to both seasons, the majority do not differ very greatly in individual frequency in the two seasons. Those which are outstandingly different, however, are:

	Summer	Autumn
Marasmius oreades	292	22
Naucoria semiorbicularis	810	82

These two together with

	Summer	Autumn
Tricholoma gambosum	397	—
Psilocybe foenisecii	110	—

form the characteristic species of the summer season.

The third class, i.e. those fungi which occur principally in the colder months of the year, contains few species and these have a relatively low frequency of individuals. The only outstanding ones are:

Naucoria melinoides, which though occurring in the autumn season, is found also in January, February, March and December.

Panaeolus campanulatus, also included in the autumn season with 50 individuals, is found in January–April with a total of 232.

TABLE 6. *List of species with seasonal frequency of individuals*

	Jan.	Feb.		Mar.			Apr.		May		June		July		Aug.		Sept.		Oct.		Nov.		Dec.		Jan.
	1	1	15	1	17	31	14	26	10	24	10	22	8	22	12	26	9	25	7	21	6	18	3	16	1
Anellaria separata	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1	.	7	.	.	.	.
Bolbitius intellinus	.	.	.	.	.	.	.	2	1	7	.	.	.	.	.	.	.	.	.	.	1	.	.	.	.
Bovista plumbea	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1	.	.	.	.
Clavaria corniculata	4	.	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	3	.	.	.
C. dissipabilis	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1	.	.	.	.
C. flaccida	2	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
C. fusiformis	3	2	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	2	.	.	.	.
C. tenuipes	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	11	.	.	.	.
Clitocybe aurantiaca v. albida	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	3	.	.	.	.	.	.	.
C. cyathiformis	6	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
C. fragrans	6	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	12	.	9	.	.
C. infundibuliformis	.	.	.	.	.	.	.	.	.	.	.	.	17	53	.	.	1	56	27	3	7	.	.	.	.
C. rivulosa	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	3	53	228	51	78	4	8	.	2
Clitopilus cancrinus	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	2	46	.	2	.	.	.	.
C. prunulus	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	3	1	.	.	.	.	.	.
Coprinus niveus	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	12	1	3	94	.	.	.	.
C. plicatilis	.	.	.	.	.	.	.	.	4	43	.	6	.	.	.	.	.	7	.	.	32	.	.	.	.
Cortinarius anomalus	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	3	3	1	.	.	.	.
Entoloma jubatum	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	3	.	.	.
E. sericeum	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	17	.	.	.	.
Galera hypnorum	.	.	1	1	.	.	.	.	1	5	.	.	.	.	.	.	2	175	209	42	232	2	.	.	.
G. tenera	.	.	.	.	.	.	.	.	.	14	1	2	.	.	.	.	.	24	2	.	91	.	18	.	.
Hebeloma crustuliniforme v. minus	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	2	.	.	.	.	.	.
Hygrophorus chlorophanus	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	2	.	.	.	.
H. coccineus	2	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	3	2	.	.	.	.
H. Colemannianus	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	3	.	.	.	.
H. ovinus	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1	2	.	.	.	.
H. pratensis	1	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1	.	.	.	.
H. psittacinus	.	5	.	.	.	.	1	.	.	.	.	.	.	.	.	.	.	.	.	.	3	.	.	.	.
H. puniceus	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
H. virgineus	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	34	1	14	2	.
H. virgineus v. roseipes	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	16	.	7	.	.
Inocybe lacera	.	.	.	.	.	.	.	.	.	5	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
I. rimosa	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1	.	.	.	.	.	.
Lepiota clypeolaria	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	14	13	3	.	.	.	.	.
L. cristata	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1	.	.	.	.	.	.
L. gracilenta	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	2	12	.	7	.	.	.	.
L. granulosa	3	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	5	.	.	2	.
L. permixta	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	3	.	.	.	.	.	.
L. procera	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	7	.	.	.	.	.	.
Leptonia lampropus	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	3	.	.	.	.	.	.
Lycoperdon depressum	2	18	8	3	2	2	.	.	.	.	.	.	.	.	.	.	1	26	6	2	6	.	.	.	.
L. nigrescens	1	2	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	4	10	15	11	.	.
L. perlatum	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	14	3	1	14	4	.	.	.
L. pusillum	.	8	8	.	.	.	.	.	.	.	.	.	.	.	.	.	.	6	.	.	6	.	.	.	.

Table 6 (cont.)

	Jan.	Feb.		Mar.			Apr.		May		June		July		Aug.		Sept.		Oct.		Nov.		Dec.		Jan.
	1	1	15	1	17	31	14	26	10	24	10	22	8	22	12	26	9	25	7	21	6	18	3	16	1
Lycoperdon umbrinum	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1	1	.	.	.	.	.
Marasmius dryophilus	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	12	19	3	.	.	.	.	.	.
M. erythropus	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	26	4	19	.	.	.	.
M. oreades	.	.	.	.	.	.	.	.	30	102	1	111	23	25	.	.	.	7	14	1	.	.	.	.	.
Mycena ammoniaca	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	34	.	.	.	.
M. avenacea	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	3	.	.	34	.	.	.	.
M. epipterygia	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	44	343	93	563	22	.	.	.
M. filopes	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	6	.	.	.	.
M. flavoalba	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	11	.	.	.	.
M. metata	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	208	.	3	.	.
M. pelliculosa	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	2	.	.	10	.	.	.	.
M. pura	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	20	29	3	.	.	.	.	.
Naucoria badipes	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	3	4	.	.	.
N. cerodes	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	9	.	.	.	.	.	.
N. melinoides	10	7	6	4	2	.	26	.	.	.	.	.	.	.	.	.	.	.	17	.	49	28	40	6	.
N. semiorbicularis	.	.	.	.	.	.	.	.	19	253	2	288	125	123	.	8	55	26	1	.	.	.	.	.	.
N. striaepes	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1	.	.	.	.	.	.
Nolania proletaria	.	.	1	.	.	.	11	.	8	15	.	.	.	.	.	.	.	1	5	.	6	.	.	.	.
Omphalia fibula	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	13	5	.	220	13	26	.	1
Panaeolus campanulatus	9	19	42	12	5	13	132	12	.	7	.	2	.	.	.	.	.	.	.	.	50	.	.	.	.
P. papilionaceus	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1	.	.	14	.	1	.	.
P. sphinctrinus	5	1	6	.	.	.	4	.	.	28	7	16	.	.	.	.	.	3	.	.	4	.	.	.	.
Peziza aurantiaca	4	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Psaliota arvensis	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1	.	.	.	.	.	.	.
P. campestris	.	.	.	.	.	.	.	.	.	.	1	1	.	1	.	.	.	8	10	33	32	8	.	.	.
P. comptula	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1	.	.	.	.
P. dulcidula	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	12	.	3	.	.	.	.	.
Psilocybe ericaea	.	.	.	.	.	.	.	.	3	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
P. foenisecii	.	.	.	.	.	.	60	27	1	8	.	60	.	14	.	.	.	.	3	.	.	.	.	.	.
P. semilanceata	1	.	.	.	.	.	2	.	.	.	.	.	.	.	.	.	.	.	117	3	324	8	10	.	.
Stropharia albocyanea	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1	.	.	.	.
S. aeruginosa	.	.	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	4	2	13	1	.	.	.
S. inuncta	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	6	6	3	.	.	.	.
S. merdaria	.	.	.	.	.	.	.	.	.	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
S. semiglobata	5	2	1	.	.	2	1	.	1	.	.	.	.	.	.	.	.	104	13	2	61	3	11	.	.
Tricholoma carneum	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	9	3	.	4	.
T. gambosum	.	.	.	.	.	.	.	62	88	244	2	1	.	.	.	.	.	.	.	.	.	.	.	.	.
T. melaleucum	.	.	.	.	.	.	.	.	.	2	.	.	.	.	.	.	.	.	.	7	41	9	2	8	.
T. personatum	2	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	3	10	21	14	.	2
T. sordidum	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1	4	.	.	.
T. terreum	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	12	4	1	.	.	.	.
T. terreum v. strosquamosum	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	9	9	.	.	.
Tubaria furfuracea	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1	.	.	.	.	.	.
T. inquilina	.	6	26	71	19	.	.	.	11	30	.	.	.	.	.	.	.	.	.	.	.	9	11	26	7

TABLE 7. *List of autumn species*

Clavaria tenuipes	11	*Mycena epipterygia*	1065
Clitocybe fragrans	21	*M. flavoalba*	11
C. infundibuliformis	94	*M. metata*	211
C. rivulosa	425	*M. peliculosa*	12
Clitopilus cancrinus	50	*M. pura*	52
Coprinus niveus	110	*Naucoria melinoides*	134
C. plicatilis	39	*N. semiorbicularis*	82
Entoloma sericeum	17	*Nolania proletaria*	12
Galera hypnorum	661	*Omphalia fibula*	277
G. tenera	135	*Panaeolus campanulatus*	50
Hygrophorus virgineus	49	*P. papilionaceus*	16
H. virgineus v. roseipes	23	*Psaliota campestris*	91
Lepiota clypeolaria	30	*P. dulcidula*	15
L. gracilenta	21	*Psilocybe semilanceata*	462
Lycoperdon depressum	41	*Stropharia aeruginosa*	20
L. nigrescens	40	*S. inuncta*	15
L. perlatum	36	*S. semiglobata*	198
L. pusillum	12	*Tricholoma carneum*	12
Marasmius dryophilus	34	*T. melaleucum*	59
M. erythropus	49	*T. personatum*	56
M. oreades	22	*T. terreum*	17
Mycena ammoniaca	34	*Tubaria inquilina*	11
M. avenacea	37		

TABLE 8. *List of summer species*

Bolbitius intellinus	10	*Nolanea proletaria*	23
Clitocybe infundibuliformis	70	*Panaeolus campanulatus*	21
Coprinus plicatilis	53	*P. sphinctrinus*	51
Galera tenera	17	*Psilocybe foenisecii*	110
Marasmius oreades	292	*Tricholoma gambosum*	397
Naucoria semiorbicularis	810	*Tubaria inquilina*	41

Tubaria inquilina is found with a frequency figure of 41 in summer and 11 in autumn but is also present in January, April and December with a total of 155.

The "peaks" in the numbers of fungus sporophores which occur in these two seasons, autumn and summer, represent a summation of the totals of the numbers of individual species. In this connexion it is found that certain species contribute very largely to these "peaks". If the autumn season is considered as lasting from 9 September to 3 December the number of species found during that period was 81 and the total number of individuals was 4903. The effect of certain individual species on this total is shown below:

Over 1000	*Mycena epipterygia*		1065	
500–1000	*Galera hypnorum*		661	
	Psilocybe semilanceata	462		
	Clitocybe rivulosa	425		3678
	Omphalia fibula	277		
	Mycena metata	211		
100–500	*Stropharia semiglobata*	198	1952	
	Naucoria melinoides	134		
	Galera tenera	135		
	Coprinus niveus	110		
Below 100	71 species with a total of		...	1225

This shows that, of the total number of species, 10 contribute approximately 75 % of the total number of individuals and the other 71 only contribute 25 %.

Similarly the effect of individual species on the totals of the summer season from 26 April

to 22 July is shown below. Here the total number of species found was 19 and the total number of individuals was 1916. The outstanding individuals are:

Over 1000	—			
500–1000	*Naucoria semiorbicularis*		810	1609
100–500	*Tricholoma gambosum*	397	799	
	Marasmius oreades	292		
	Psilocybe foenisecii	110		
Below 100	15 species with a total of			307

The influence of individual species on the total is even more striking in this case, as the four species listed account for about 85 % of the total number of individuals.

The "out of season" fungi show the same trends, but to a less marked extent. Fewer species are present to any high degree of frequency: e.g., out of a total of 656 individuals found during the "out of season" period only two species have a relatively high frequency:

Over 1000	—		
500–1000	—		
100–500	*Panaeolus campanulatus*	232	387
	Tubaria inquilina	155	
Below 100	27 species with a total of		269

In this case the two more abundant species only contribute about 60 % of the total number of individuals. This again emphasizes the fact that less favourable periods are characterized by a comparatively large number of species with a comparatively small number of individuals while, in the favourable periods, the converse is true.

5. Conclusions

The main point made by this work appears to be that the seasonal variation of the fungi of grasslands is conditioned by environmental factors, and it is reasonable to assume that probably the same factors are responsible for the seasonal variation of fungi in any other habitat or vegetation community. The point that a mycological flora can only be described as "characteristic", either of time or place, by due consideration of the frequency of individuals, receives additional emphasis as the work proceeds. Though certain conditions must obtain in order that fungus sporophores may be produced in abundance, it may well be that a rapid increase in numbers is determined or influenced by the conditions which were in force prior to such increase. For instance, the rapid rise in sporophore numbers in the autumn season may have been caused by the previous high maximum temperatures, so confirming the generally held opinion that a hot summer is invariably followed by a prolific autumn fungus season if the autumn conditions are themselves favourable.

Some work has been done on the temperature relations of certain fungi in culture with the object of determining whether those that flourish in the warmer months have a higher optimum temperature, or a greater toleration of high temperatures, than those which grow in the cooler months. In general, it seems that this is the case though the difference in the optima is, at most, only a few degrees and, as the results are not conclusive, they are not included here.

The above type of investigation is at present being applied to the fungi of certain woodlands.

6. Summary

1. This paper deals with the seasonal distribution of grassland fungi and attempts to show correlation between sporophore production and environmental conditions.

2. The experimental methods of recording the factors of temperature, rainfall, soil-water content and hydrogen-ion concentration of a given area on each of three grassland stations for a period of 12 months, have been described.

3. Lists are given showing the seasonal distribution of the fungi on the three areas.

4. The relation between the factors of temperature and water content of the soil and the numbers of fungus sporophores throughout the year has been examined critically and it is suggested that the summer and autumn fungus seasons are conditioned by these factors.

5. An alphabetical list of species showing seasonal frequency of individuals is given.

6. The seasonal distribution of individual species of fungi and the effect of these species in determining the mycological floras characteristic of the summer and autumn seasons is discussed.

7. There is a brief discussion of results.

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REFERENCES

Baker, H. & Clapham, A. R. (1939). Seasonal variation in the acidity of some woodland soils. *J. Ecol.* **27**, 114.

Wilkins, W. H., Harley, J. L. & Kent, G. C. (1938). The ecology of the larger fungi. II. The distribution of the larger fungi in part of Charlton Forest, Sussex. *Ann. appl. Biol.* **24**, 703.

Wilkins, W. H. & Patrick, Sheila H. M. (1939). The ecology of the larger fungi. III. The constancy and frequency of grassland species with special reference to soil types. *Ann. appl. Biol.* **26**, 25.

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THE INFLUENCES OF SOWING DEPTH AND MOISTURE ON SMUT DISEASES, AND THE PROSPECTS OF A NEW METHOD OF CONTROL

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(With 11 Text-figures)

Part I. Effect of methods of planting on smut diseases, and analysis into factors

(1) *Introduction: The method of planting effect*

During the course of seed disinfection experiments with flag smut of wheat an experiment was repeated the next season in another locality and it was found that the supposedly exact repetition gave much less disease than the first. As a similar result had happened a few years before with covered smut of barley (Jones, 1934), the histories of both pairs of field experiments were reviewed and it was found that repetition was not exact but differed in method of planting.

Wheat and barley under perennial irrigation in Egypt are either planted by the *herati* method of broadcasting seed on moist land and ploughing it in, or else by the *afir* method of broadcasting on dry land, harrowing in the seed with a baulk of wood and irrigating. The farmer's choice of method is usually dictated by conditions mentioned later. Examination of pairs of wheat fields in which all other factors were said to be the same, but in which both methods of planting were used, showed that *herati*-sown plots consistently gave more flag smut than *afir*-sown plots. Later experiments showed that there was two to three times more flag smut in *herati*-sown plots, and similar tests with barley proved that the *herati* plots were six times more heavily attacked by covered smut than the *afir* plots, thus explaining satisfactorily the result. Later still, it was found that bunt of wheat and grain smut of millet and broom corn were almost equally sensitive to difference in method of planting, and the *ad hoc* flag smut investigations thus broadened into research on the soil influences operating at planting time upon seed-borne cereal smuts.

(2) *Possible causes of the method of planting effect*

The most obvious difference between the *herati* and *afir* methods of planting is in soil moisture, the *herati* plots being only moist enough for good ploughing, while *afir* plots, being irrigated immediately after sowing, are wet. A second difference lies in the depth at which the seed is planted. In *herati* plots the seed is covered with a plough set at 12–15 cm. depth, so that the seed is buried at any depth between the surface and the plough sole: seeds which happen to be left near the surface fail to germinate owing to lack of moisture, while the most deeply buried seeds germinate poorly, so that the average depth of effective

planting is about 8 cm. In the *afir* plots the seeds broadcast on the surface are covered by harrowing with a wooden baulk while the land is dry, and are thus buried only about 4 cm. deep before they are irrigated.

While these two factors of depth and moisture have been proved to result in large and constant difference in disease and thus adequately explain the effect of method of planting, it is convenient here to judge the effect of other differences between *herati* and *afir* plots. Measurements at planting time show that *afir* plots are always cooler, by a fraction to 2° C. according to season, than adjacent *herati* plots, the difference in temperature lessening until it is negligible 10 days after planting. As most of the present experiments were done when temperature was near the maximal limit for disease development, cooler plots would thus tend to develop more disease. There are also differences in temperature at various depths of soil which are rather differences in daily range than in means; these are small in proportion to the wide range of temperature which allows of disease development and seem unlikely to cause large effects. Differences in temperature between *herati* and *afir* soils could not cause the large differences observed in disease, but would rather tend to obscure them.

Experimental details for analysis of the method of planting effect

Throughout the investigation of all four diseases on five hosts the following standard method of experiment was used.

Eight rectangular blocks each 8 m. wide and usually 45 m. long, separated by 1 m. on the long side, were chosen at random for moist (*herati*) and wet (*afir*) treatment. Each block was again divided lengthwise by small ridges into four strips, 1·5 m. wide, chosen at random, for planting near the surface (0·5 cm.), 4, 8 and 12 cm. deep.

The land was dry when divided, but 20 days before the planting date all the strips in four of the blocks were irrigated and allowed to dry so that by the normal date of sowing they were in a suitable condition of moisture for normal *herati* sowing. The seed was inoculated by adding 1 per 1000 by weight of smut spores.

In each strip five long rows were sown with a small drill, the tines previously being fixed rigidly at the correct depth. Seeds sown nearest the surface (0·5 cm.) were drilled on the surface and afterwards covered by hand with soil of the same plot. Drilling could not be used for millet and broom corn; five ridges were made running the length of each strip and their tops levelled: seeds were then sown in holes made by special dibbles for each depth, or sown on the surface and just covered for the 0·5 cm. sowing. In all experiments, seed for sowing the 0·5 cm. moist plots was soaked in water for 24 hr. before sowing date, as otherwise it would not have germinated.

Immediately after drilling, the dry plots, containing about 8 % moisture[1] were irrigated, as in the *afir* method of sowing, the moisture content of these now wet plots being thus raised to about 32 %. The moist plots (corresponding to the *herati* method of sowing) at this time contained about 25 % moisture. After this, both the moist and wet plots were given the ordinary irrigation and manurial treatment which the crop receives in farming.

Germination, or more strictly emergence of seedlings, could not be counted, but was assessed relatively to other plots. In some cases, such as the Hindi 61 wheat used for flag smut, there were marked differences in the appearance of seedlings, the surface-sown seedlings being dark green with broad, sturdy leaves, the 4 cm. sowings green with longer leaves, the 8 cm. sowings yellow with long, narrow, weak leaves, and the 12 cm. sowings orange with excessively long, narrow and weak leaves. The coleoptiles of the deeper planted seedlings often failed to reach the surface and ruptured below, exposing the leaves which were often held at their tips by the moist soil, continued to grow at their bases, and eventually appeared above the surface in an arched form. In the other experiments the differences in appearance of seedlings planted at various depths were less marked.

Disease on each plot was counted for bunt and covered smut of barley by pulling up all plants at

[1] The authors are indebted to the Chemical Section of this Ministry for these estimations, which are percentages of weight of the moist soil.

harvest time and counting into healthy and diseased. In flag smut the disease was counted by pulling at four periods, when 81, 103, 128 and 149 days old, with similar results, presented in total, because if the plants are left till harvest time diseased plants tend to die, dry and disappear, giving an inaccurate measure of disease. In millet and broom corn the plants were not pulled up, but were counted as they stood and they were afterwards cut back to produce secondary heads, as described later.

Statistical significance and reliability of results

The results are presented in Figs. 1 and 2. The differences in all curves between depths and soil-moisture status are statistically highly significant. The differences between the pairs of curves for wet and moist soils are overwhelmingly significant, when tested by the

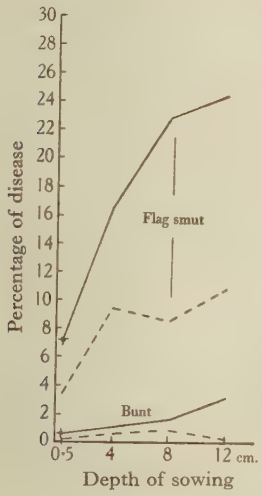

Fig. 1.

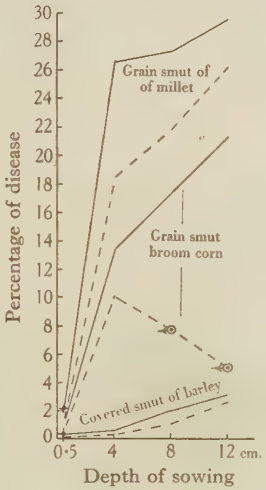

Fig. 2.

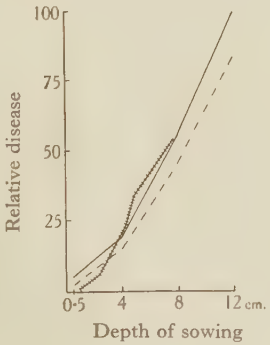

Fig. 3.

Fig. 1. Percentage of flag smut and bunt plotted against depth of sowing in moist and wet soils. —— Moist soil (*herati*); – – – wet soil (*afir*).

Fig. 2. Percentage of covered smut of barley, and grain smut of millet and broom corn, plotted against depth of sowing in moist and wet soils. + Seeds soaked for 24 hr. before planting; ⊲⊙ Seeds planted too shallow.

Fig. 3. Relative amount of covered smut of barley plotted against depth of planting in moist and wet soils in Egypt, compared with Taylor & Zehner's (1931) results in the United States. – – – *Afir*, Egypt; —— *herati*, Egypt; ····· Taylor & Zehner (1931) (U.S.A.).

X^2 method: the drop in the deepest planting in wet soil for bunt would indeed have been highly significant even if the curve had continued level.

All seed planted near the surface of moist land was presoaked in water for 24 hr., and the figures of disease from these plantings are specially marked, as they are not precisely like the others. The depths of planting were accurate when tested, as far as it was possible to measure and check small depths in soil, except that the 8 and 12 cm. plantings of broom corn in wet land were found on checking to be too shallow: it appears that dry soil fell into the dibble holes before sowing and irrigation; these two depths are also queried, and it should be noted that they minimize any observed effect.

The number of plants ultimately obtained from these sowings, and hence the reliability of each point on the graph, varies as a result of good or bad stand of plants. In covered smut

of barley the least number of any treatment was 26,000, but the proportion of diseased plants was small: in bunt the average was about 12,000, except for the deepest planting in wet soil, which was 5000, and the proportion of disease was again small. In flag smut the average was 5000, and although the 0·5 cm. planting in moist soil and the 8 and 12 cm. plantings in wet soil were only 2500, the proportion of disease was much higher than in either bunt or covered smut. In millet the number was about 550 plants throughout, and in broom corn about 750 throughout, with high proportion of disease.

Part II. Discussion of causes

(1) *The influence of depth of sowing*

In all the curves in Figs. 1 and 2 there is an obvious progressive increase of disease with each increase of depth, with only three exceptions in thirty, all occurring in deeper plantings in wet soil.

The almost constant influence of depth on these four similar seedling infecting smut diseases is more remarkable when one considers that they differ in respect of possible source of infection. In covered smut and grain smut the only recorded source of infection is seed-borne spores: in bunt this is usually the case, but infection may exceptionally come from soil-borne spores, while in flag smut it is well established that there are two sources of infection—from seed and soil.

For each disease the pair of curves for moist and wet soil may in general be considered together, but we shall study the effect of depth in moist soil particularly, because this is a more normal condition for growth and has given consistent results; afterwards we shall discuss the less regular curves for wet soil (in which all three exceptions to the depth of planting effect occur) when considering the soil-moisture effect. It is most convenient to study the effect of depth on each disease separately, and discuss the general relation later.

(*a*) *Covered smut of barley.*

The infection process in covered smut cannot begin until two preliminary processes are completed. First, the barley seed must have absorbed water, pushed out the coleorhiza and exposed the coleoptile, a process which normally requires 3 days under Egyptian conditions. Secondly, the smut spore must germinate, send out a promycelium and produce sporidia and infection tubes, a process which occurs more rapidly with *Ustilago hordei* than with most smuts and may be completed within 48 hr. at usual temperatures. Both host and parasite would then be in a condition to allow the infection process to begin 2–3 days after sowing. Infection is no longer possible, according to Appel & Gassner (1907) and Schellenberg (1924) after the coleoptile has ruptured and the first green leaf has pushed 1 cm. beyond it.

Seeds planted at 0·5 cm. depth in wet soil take 3 days to produce coleoptiles which start rupturing on the fourth day, so that little more than a day is available for infection to become established. Moreover, in these shallow plantings, most of the coleoptile has been growing in air which, for several hours at least, is probably too dry to retain sufficient water film to keep the infecting fungus alive. Under these conditions the chances of infection seem very slight.

Faris (1924*b*) found some indication that disease development depends upon environ-

ment late in the susceptible period, and even after what has hitherto been considered the susceptible period has ended, for some biologic forms of *U. hordei*, at least. He divided a lot of 7-day-old inoculated seedlings, keeping half in the greenhouse, where they became diseased, and half in the field where they grew healthily. Tapke (1938) confirmed this by a similar experiment; those plants set out-of-doors immediately after emergence developed only 27·8% of disease, while those kept for a fortnight in the greenhouse before planting out developed 55·1% and those kept for a month 65·3% of disease. Tapke found no such differences when the seed was inoculated by the spore-suspension method, which lodges the spores within the hull and apparently enables the smut to become sufficiently well entrenched in its host to resist adverse conditions after emergence, and he suggested that the hyphae from superficially dusted spores may be delayed in penetrating. However, superficial spores are the normal condition in nature, and it seems likely that for very shallowly planted seedlings penetration may not only be delayed but even prevented by adverse conditions occurring about the stage of emergence.

Seeds planted 4, 8 and 12 cm. deep similarly produce coleoptiles in 3 days, but the seedlings only reach the surface and rupture their coleoptiles when 5, $6\frac{1}{2}$ and 7 days old, thus allowing 2, $3\frac{1}{2}$ and 4 days for completion of infection: almost all this time they have been growing in soil damp enough to keep the infecting fungus alive. Chances of infection seem greatly increased as depth of sowing, or time of exposure of coleoptile to infection under damp conditions, increases, i.e. as the susceptible period of the seedling is lengthened.

This probability seems to be strengthened by the interesting behaviour of hull-less seeds to disease. Tisdale (1923) noticed that plants from dehulled barley seed always developed more disease than plants from normal seed planted at the same depth, and thought that the hulls were the main obstacle to infection. Faris (1924*a*) confirmed this observation, but noticed that seedlings from dehulled seed grew twisted and reached the surface very slowly: he also thought that the greater development of disease might be due to removal of the hull obstacle. Tisdale & Tapke (1924) working with *U. nuda* (which, however, they found to be affected by surface seed disinfectants and was therefore more likely to be the then unrecognized *U. nigra*) again confirmed the higher susceptibility of seedlings from dehulled seed, together with the twisting of such seedlings, which increased with deeper sowing and which they considered to be caused by the fungus. However, experiments in Egypt (Jones *et al.* 1939) prove that the twisting of dehulled seedlings occurs even in the absence of smut spores, and is apparently due to functional derangement connected with a very rapid intake of water by the hull-less seed. Even in normal seed with hulls, very rare cases can be found in which the coleoptile bursts at the base as well as rupturing at the apex, and this tendency seems to be greatly increased by dehulling the seeds, and again increased if spores of *U. hordei* are present on the seed: some coleoptiles still rupture normally at the apex, but in the majority the coleoptile bursts prematurely at the base, as if by internal pressure and weakness of the coleoptile wall. If soil pressure is removed and the seeds are grown in damp, dark air such abnormal premature bursting at the coleoptile base very rarely occurs.

The dehulled seed produces a coleoptile in 2 days instead of 3, and it takes longer than usual for its twisted coleoptile or leaves to reach the surface. The seedling is subjected to infection both earlier and longer than normal coleoptiles, and this may be another cause of more disease in seedlings from dehulled seed.

Taylor & Zehner (1931) have found that not only covered smut but also loose smut of

barley are increased by deeper planting. Their results for covered smut are shown in Fig. 3, together with the Egyptian results with which they agree very closely, the graph being drawn with the maximum disease equal to 100 and the other percentages in proportion at their proper depths. These authors do not suggest any cause for the increase of disease with depth.

With regard to loose smut, Taylor & Zehner's result appears surprising, as it is scarcely to be expected that an internal infection like loose smut would be greatly influenced, if at all, by external conditions at planting time. At this time, however, it was not realized that there was a "loose smut" caused by *U. nigra*, a seedling infection, as well as the true loose smut caused by *U. nuda*, a flower infection. Tapke (1935) has reviewed the irregular results of seed disinfection with loose smut in the United States, and concludes that they were due to confusion between *U. nuda* and *U. nigra*. Moore & Allison (1935) have shown that *U. medians* is widespread in the United States, and the situation is still further complicated by Ruttle's report (1934) that there are five intermediate types between *U. hordei* and *U. nuda*, some of which resemble *U. nigra* and *U. medians*. It is therefore necessary that both *U. nuda* and *U. nigra* should be retested for response to depth of planting.

(*b*) *Grain smut of millet and broom corn.*

Sphacelotheca sorghi causes grain smut both on millet and on a variety of millet called broom corn: in these experiments one source of spores was used for the artificial inoculation of both hosts. The disease is wholly seed-borne, and its relationship to depth of planting might be similar to covered smut of barley. The results show a larger and quicker response.

Under tropical conditions the millet coleoptile may appear in 1 day and grow to 2·5 cm. in 2 days in damp soil, while in drier soil Kulkarni (1918) showed that growth is much slower. At this early stage the mesocotyl begins to extend rapidly into a rhizome which may grow more than 1 cm. within 2 days, the growth rate depending on moisture and the length being ultimately checked by light. Thus the young millet coleoptile node is soon pushed above soil surface, while in wheat and barley the rhizome only begins to grow several days after the coleoptile has ruptured and the susceptible period has ended, and it remains below the surface at tillering level.

Germination of the spores of the parasite is visible in 6 hr., and within a day promycelia and sporidia may be produced: Kulkarni (1918) found infecting hyphae most abundant on the upper part of the mesocotyl, near the coleoptile node.

It is clear, then, that although both host and parasite may quickly become ripe for infection, the mesocotyl also may grow so rapidly that in a few hours it may push the susceptible area of shallowly planted seedlings above the soil surface into comparatively dry air where chances of infection seem unfavourable. Small differences in the relative rates and duration of these three rapid processes may then greatly alter the amount of disease developed. It is possible that the amount of disease in shallow plantings might be appreciably affected by the hour of planting, for if coleoptiles emerged early in the morning, light and comparative dryness of air would check growth of the mesocotyls, while if the coleoptiles emerged in the evening, darkness and humid air would encourage growth of the mesocotyls. In any case, on the third day the coleoptiles rupture and the susceptible stage ends.

More deeply planted seedlings will certainly have their coleoptile nodes exposed to damp

soil for many hours longer than those surface sown, while the rapidly growing mesocotyl is forcing the coleoptile node through the 4, 8 or 12 cm. thick layer of soil to the surface. This is probably time enough to allow such a rapidly developing parasite to complete infection, and thus explains the great increase of disease from 1·5 to 1% in plantings near the surface, to 26·6 and 13·4% in plantings at 4 cm.: further increases of depth do not cause much increase in disease.

It is always possible in smut diseases that some "latent" infection may not manifest itself in disease, is not counted, and may therefore give a misleading estimate of how much infection has actually occurred. One of Brefeld's (1905) experiments was therefore repeated by cutting down all the plants to one-third of their height after they had produced heads, and allowing them to grow secondary heads. In nearly all cases, previously diseased plants continued to give secondary diseased heads, and previously healthy plants produced healthy secondary heads. But there were a few exceptions: 0·5% of previously healthy millet and 0·4% of broom corn plants became diseased, and 2·2% of previously diseased millet plants and 0·8% of broom corn plants gave healthy secondary heads. Six of these millet plants and two of broom corn gave a mixture of diseased and healthy secondary heads on the same plants. These small irregularities did not appear to be related to depth of sowing or soil moisture. Melchers (1933) has carried out interesting experiments on latency in this disease.

In this experiment latent infection was not important, and the figures for disease may be taken as almost exact figures for infection.

(*c*) *Bunt of wheat.*

The causal agent of bunt, dealt with in these experiments, is *Tilletia foetens* (Berk & Cust.) (= *T. laevis* Kuhn).[1]

The case of bunt may not be so simple as the two previous diseases because infection may come from spores in the soil as well as on the seed. Appel (1924) reported a case from Germany where wind-blown spores from threshing operations seem to have caused disease in a neighbouring field, and in the United States Flor (1933) reported soil infection but stated that only Form *T'*, of *T. tritici* was concerned, *T. laevis* not being important as a soil infecting agent. Soil infection seems unlikely in Egypt because threshing is always done just after harvest, leaving an interval of 5 months of hot weather, while the land is constantly irrigated for summer crops, until sowing time. The efficient control achieved by seed disinfection (Jones *et al.* 1939) seems to prove that seed is the only source of infection in Egypt.

The germination of spores of *Tilletia tritici* is slower than those of *Ustilago hordei*: according to Prévost, Tubeuf and Volkart, quoted by Woolman & Humphrey (1924), the optimum temperature is between 16 and 18° C. and requires $2\frac{1}{2}$–3 days. This was approximately the temperature of the soil in our experiment, and if a short time is allowed for formation and fusion of sporidia, it would be possible for wheat to be attacked soon after the coleoptile appears.

Sartoris (1924) found that wheat is most liable to attack by *Tilletia tritici* during the first 3 days after planting, gradually becomes less liable until the ninth day, then rapidly becomes

[1] Identification kindly made by the Imperial Mycological Institute.

resistant until it is completely immune on the thirteenth day, which was about the stage when the coleoptile ruptured and green leaves appeared. His figures are reproduced as a graph in Fig. 4. Care is needed in applying these facts, since the period measured in days will change with external conditions and they are more truly stages in the development of the plant. The conditions and results of his experiments do not occur under, and must not be directly applied to, natural conditions. Sartoris applied sporidial inoculum to the whole plant at definite ages, while in nature inoculum would be present, usually as dry spores, from the moment of planting throughout all stages of the development of the plant, and not necessarily over the whole surface of the plant.

Woolman (1930) pointed out the successive obstacles which have to be overcome before infection by *T. tritici* is fully established, leaving room for a large element of apparent chance even when conditions are favourable. Thus, provided spores germinate well and the plant is in the susceptible stage, penetration of the coleoptile occurs with equal facility in both resistant and susceptible varieties, no fewer than 100 points of entry being found. Penetration only proceeds to the second phase (growth of mycelium in the deeper layers and change in staining reaction from Gram-negative to positive) in susceptible varieties, and even here there seems to be strong inhibition, for never more than four, and rarely more than two, areas of second-phase infection were found. That is, never more than 4%, and usually only 2% of penetrations ultimately result in established infection. It is interesting that three-fourths of the points of attack were confined to the lower half of the coleoptile and occurred in groups, although the infection was in this case evenly distributed in the soil.

There are certain good records of the stage at which penetration begins. Sartoris (1924) observed entry 6–8 days after planting inoculated seeds on the surface of moist soil at about 30° C., or, if germinated spores are placed on 2–3-day-old seedlings, entry can be observed 2–3½ days later, that is on 4–6½-day-old seedlings. Woolman (1930) working at 15° C., a more suitable temperature for sowing wheat, planted seeds 5·5 cm. deep in inoculated moist soil and found two to three points of entry on every seedling at time of emergence. The age of the seedlings is not stated, but from his previous experiments it may be estimated at 10 days; seedlings 7 days older generally bore no fewer than 100 points of entry. He concluded that infection begins at about the time of emergence of seedlings, but that this may not be true in all circumstances. Both Sartoris and Woolman showed that there is some resistance to infection by the host at this stage of development.

The soil temperature in our depth of planting experiment was a few degrees higher than in Woolman's experiments, and about half the final number of seedlings in the surface plantings had emerged and ruptured their coleoptiles after 9 days, thus scarcely allowing time for penetration before the susceptible period ended. One and a half days longer were required for half the coleoptiles of the 8 cm. plantings to emerge and rupture, while in the 12 cm. planting emergence and rupture of coleoptiles was so sparse and prolonged that it was difficult to estimate: it is to be expected that the additional time would be increasingly favourable for infection by bunt.

(d) Flag smut of wheat.

The problem of flag smut is more complex than in the three previous diseases, partly because of the peculiar germination conditions required by the spores, and partly because

a new complication is introduced by the known possibility of infection from spores in the soil.

In the United States and Australia it would seem that soil infection is often negligible in amount because seed disinfection frequently controls disease almost perfectly. In Egypt such efficiency has never been approached with any of a large range of disinfectants (Jones *et al.* 1939): the copper sulphate dip advised by Morwood (1931), for example, never prevents more than half the disease under the *herati* planting method, and is only slightly more efficient under the *afir* method of planting.

Soil infection must then be responsible for about half the disease in Egypt. Perhaps this heavy soil infection is explained by the Egyptian method of winnowing chopped straw and grain by tossing them into the air on windy days so that flag-smut spores are widely spread, that these spores may remain viable for 11 years in relatively dry air (Noble, 1934) which is usual in Egypt, and that flag-smut spores do not germinate easily when merely wetted.

Noble (1923) found that flag-smut spores only germinate after 3 days' soaking in water, and then slowly unless young wheat or other cereal tissue is added to stimulate them into greater activity. Stimulation is not so marked when spores and tissue have been placed in contact from the beginning: Noble (1924) suggested that this might be due to rapid loss of some volatile agent which could only penetrate pre-soaked spores, and he imitated the stimulus given by fresh cereal tissue with essential oils, benzaldehyde, etc.

The special needs of spore germination may partly explain the unusual shape of the disease-depth curves in flag smut. In infected dry soil, irrigated immediately after planting, 3 days must pass before seed or soil-borne spores begin germination, and as spores and stimulating host have been in contact throughout, presumably only a few spores germinate. The time needed for infection is not known, except that Noble (1924) never observed infection if seedlings longer than 4 cm. were inoculated with dry spores, though infection occurred with germinating spores. But as the coleoptiles of shallowly planted seeds begin to emerge on the fourth day and rupture soon afterwards, it is clear that very little time is available for infection in the 0·5 cm. plantings; deeper plantings are allowed more time for infection and also have greater chances of meeting new inoculum as they grow through the soil, and may well become more diseased. Miller & Millikan (1934) observed increase of flag smut with increased depth of planting in Australia.

The plantings in moist soil met different conditions. Seed-borne spores began to germinate slowly after 3 days, as before, but the soil-borne spores were already in the sensitive pre-soaked condition. Twenty-one days before sowing date (in farming practice 2–3 weeks according to soil) the plots were irrigated and allowed to dry until moist enough for ploughing. This irrigation pre-soaked the spores in the soil, rendering them receptive to stimulation for 28 days (Noble, 1923). Then as soon as wheat was planted among this sensitized inoculum it would at once stimulate soil spores into much faster germination. In addition, it is probable that the host itself has less resistance at this early stage than later when it is near the end of the susceptible stage. The expected result would be more disease in plantings made in moist soil than in plantings in dry soil, irrigated at planting time. Actual results show a difference in disease, a greater difference than is generally found in the other diseases between moist and wet soils.

(2) *A theory for the influence of depth*

Faris (1924*a*) pointed out that a separate analysis of each individual factor in smut production is impossible: there are numerous factors, with different favourable ranges and optima, acting and interacting on the host, the parasite and the disease. In experimentation an attempt is made to maintain all factors but one constant, but in nature successive stages of infection may demand different conditions. It is rare in experiments for all plants to become diseased, and Faris's highest figure of 97 % could only be obtained by three changes of temperature during the course of the infection experiments..

Thus, in studying the influence of depth, other factors may have operated and their effects been included in the observed results. One important assumption made here is that the final amount of disease observed corresponds to the infection occurring in the seedling stage, so that there is no "latent" or "suppressed" disease. Latent disease was tested in grain smut of millet and broom corn only and found to be negligible. This distinction will be maintained by referring to "infection" in the theoretical treatment, and to "disease" in the actual observations.

The effect of depth is generally large, and is consistent enough to support a theory which may be capable of being tested.

The theory that varietal resistance to smuts may be correlated with length of susceptible stage, or speed of germination or emergence, has frequently been disproved, e.g. by Reed & Faris (1924) for covered smut of oats, and for both grain and kernel smut of millet, where varietal resistance must therefore be innate. It has been suggested in the previous discussion that the increase of disease with depth might be explained as a lengthening of the susceptible stage of the seedling. Faris (1934) tested the effect on disease of the length of the susceptible stage by sorting the slowly and quickly growing seedlings within each of two varieties, and found that the rapidly emerging seedlings were 40 % more susceptible to attack by *Tilletia laevis*; a similar but less striking result was obtained with *T. tritici*. However, this does not really test the theory, for if external conditions were exactly the same for all seedlings, and yet wide variation in time of emergence occurred, it can only be concluded that there were either great differences in size or quality of seed or else genetic differences which are likely to be associated with different resistance. Faris does not claim that these experiments disprove the theory, but remarks that conditions favouring the host also appear to favour disease, as in the symbiotic relationship of rusts. We shall later give evidence pointing to a contrary tendency towards early destructive parasitism and death of the host plant when grown under extreme conditions.

It is known from the work of Sartoris (1924) that infection with bunt is easier on very young seedlings than on old, and his figures for bunt are shown graphically (Fig. 4, curve *a*). By planting deeper and deeper, without any sorting into quickly or slowly growing seedlings and by keeping all under the same conditions, average emergence and rupture of the coleoptile occurs later and later, so that Sartoris's curve needs to be lengthened at all points, i.e. the time in days would be lengthened to accord with stages in development of the seedlings, as shown diagrammatically in Fig. 4, curve *b*.

We may assume that the effect of depth of planting is due wholly to the lengthening of the susceptible stage of the seedling, i.e. to the time during which the seedling is exposed to infection, and endeavour to foresee the consequences.

In the simplest cases, where only seed infection occurs, and assuming that all external factors are favourable for infection, the time needed for infection to be established will not be the same for all individual plants, but will vary from seedling to seedling and spore to spore. The simplest assumption is that the variation will be of a normal type, and the

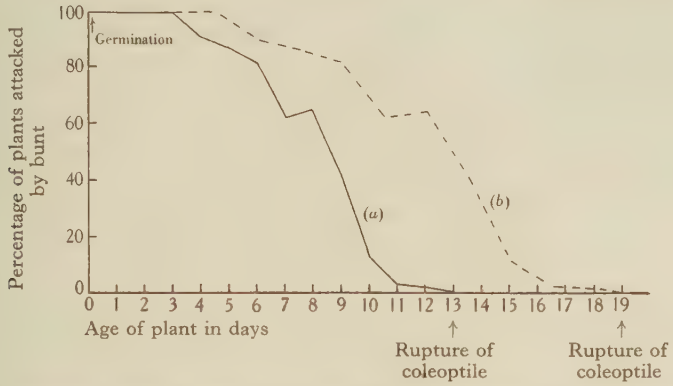

Fig. 4. Graph *a*: percentage of plants attacked by bunt after being subjected to sporidial infection at various ages (after Sartoris's (1924) figures. Graph *b*, probable diagrammatic representation of the same curve under slower growth conditions.

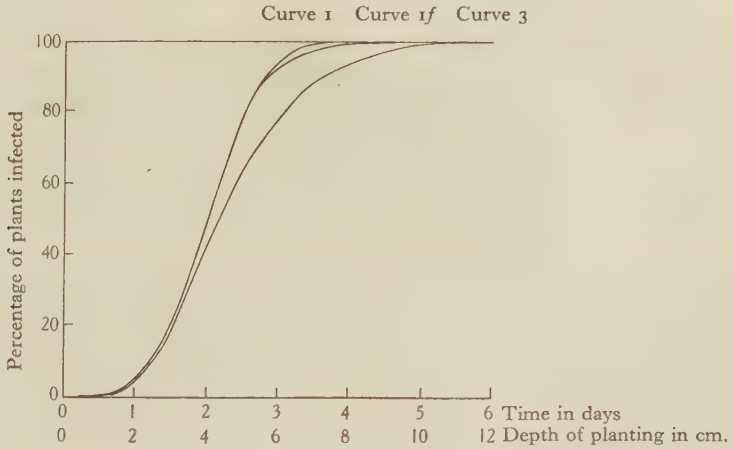

Fig. 5. Diagram showing the normal survivor curve (curve 1) postulated for infection-time relation, modified to allow for increasing resistance of the host with age (curve 1*f*), and translated into infection-depth curve (curve 3).

curves of percentage infection plotted against time of exposure to inoculum will be the sigmoid curve of normal variation or survivor curve (Fig. 5, curve 1).

The time needed for every plant to become infected is not known, but let us assume that 4 days are needed between the first and the last infection. This simple curve of normal variation must be modified, because Sartoris has shown, at least for bunt, that the seedling becomes gradually more resistant to infection with age; the later part of the curve must therefore be drawn out laterally, as shown in Fig. 5, curve 1*f*.

The curve 1*f* will not serve for percentage infection plotted against depth, because deeply planted seedlings grow relatively faster than those shallowly planted by an amount which can be measured for each host under specified conditions. In order to translate an infection-time curve into an infection-depth curve, the upper parts of the curve must again increasingly be drawn out laterally by this amount and added to curve 1*f*, giving Fig. 5, curve 3. This represents a possible theoretical curve of depth of planting against percentage infection, assuming that all plants become infected after 4 days' exposure to inoculum, or by being planted at 12 cm. depth.

The time or depth factor in infection may, however, operate at different rates from those assumed. Instead of requiring 12 cm. depth or 4 days for completion, it might operate

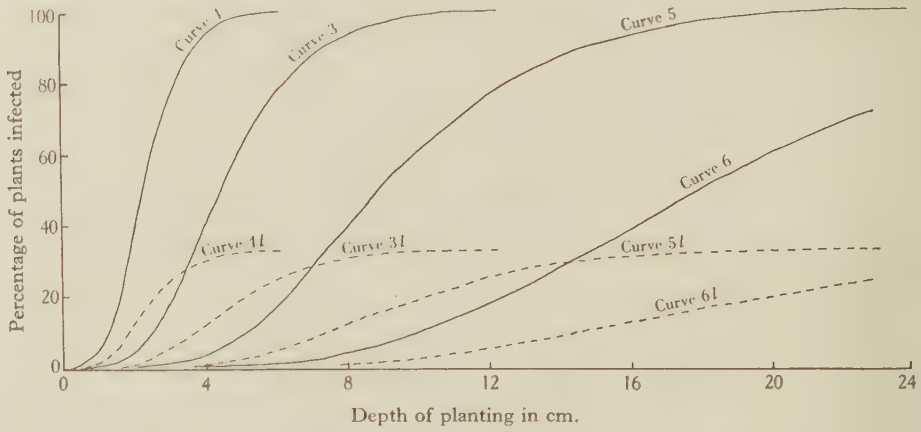

Fig. 6. Showing a family of infection-depth curves 4, 3, 5 and 6, made by deforming the ordinates of the first infection-depth curve (curve 3), and the derived curves 4*l*, 3*l*, 5*l*, and 6*l* made by reducing the originals to one-third.

rapidly and only need 6 cm. depth (Fig. 6, curve 4), or it might act slowly and require the equivalent of 8 days in depth (Fig. 6, curve 5) or operate still more slowly and require the equivalent of 16 days to complete infection of all plants (Fig. 6, curve 6).

However, it is unusual for conditions to be so favourable that every plant becomes infected; unfavourable conditions for infection are manifested not by gradual effects on individual plants but by difference in the proportion of plants infected. Suppose, then, that all conditions remain favourable, but that one, e.g. soil acidity, is so unfavourable that only one out of three plants becomes infected. Then all points on the sigmoid curves will be reduced to one-third, giving, according to the rate of infection, curves 3*l*, 4*l*, 5*l* and 6*l*: these final curves represent a series of theoretical infection depth of planting curves controlled by an unfavourable factor. They are regraphed as four-point curves in Fig. 7.

Under field conditions all factors are varying and interacting, causing irregularities: the series of disease-depth curves observed are graphed in Fig. 8 for comparison with the theoretical series in Fig. 7.

Flag smut needs separate consideration. If seed-borne infection only occurred, the infection-depth curve would be normal, such as Fig. 9, curve 3: but, in addition, the

seedling meets soil-borne spores as it grows through the soil and this would increase infection proportionally to depth of planting.

The condition of the soil-borne spores seems more important. In dry soil, wetted immediately after planting, the soil-borne spores germinate like the seed-borne spores, and the infection-depth curve will be another curve 3: since half the disease comes from soil infection, the total of both curves is Fig. 9, curve 7, which ends at the 200% point where every seedling is infected twice, once from seed and once from soil-borne spores. Assume

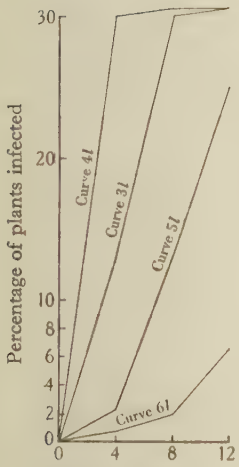

Fig. 7.

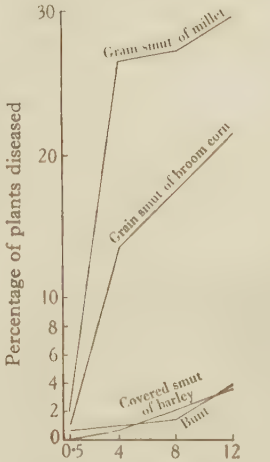

Fig. 8.

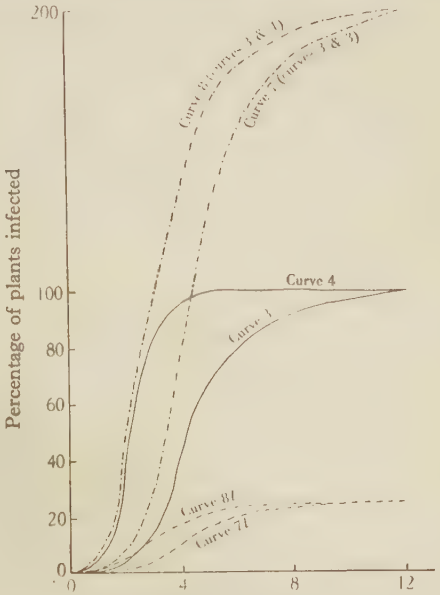

Fig. 9.

Fig. 7. A family of infection-depth curves 4*l*, 3*l*, 5*l* and 6*l* redrawn in a four-point graph to show possible theoretical infection-depth graphs.

Fig. 8. Actual disease-depth graphs obtained in the experiments in moist soil (*herati* condition) for comparison with Fig. 7.

Fig. 9. Diagram of infection-depth relationship for the special case of flag smut of wheat.

now a controlling factor giving one chance in four and the curve 7*l* is produced by dividing each point on the curve by 8 (i.e. divide by 4 and again by 2 since only single infection is needed to achieve disease).

But in moist soil the soil-borne spores are in the sensitive pre-soaked condition and will germinate ten times as fast as spores in previously dry soil when the wheat is sown near them. We may assume conservatively that soil infection would proceed twice as fast as before so that the infection-depth relationship would follow Fig. 9, curve 4. Seed and soil-borne infection will now be represented by curve 3 plus curve 4, giving Fig. 9, curve 8. Assuming the operation of the same controlling factor as before we then have Fig. 9, curve 8*l*.

When curves 7*l* and 8*l* are regraphed in four-point curves they give Fig. 10, curve 8*h*, an infection-depth curve in moist soil (*herati*) and curve 8*g*, in dry soil irrigated after plant-

ing. As wet soil always decreases disease increasingly with depth, the curve 7*g* must be further modified to give an estimated curve 7*a* which is an infection-depth curve for planting in dry soil, afterwards irrigated (*afir*). The observed disease-depth curves are set out in Fig. 11 for comparison.

It should be noted that both theoretical and actual curves for flag smut in moist soil are markedly concave in their lower halves, unlike the other theoretical curves so far examined, which are convex. The first half of a survivor curve must necessarily be convex, and similarly with the four-point graph derived from it: however, a modified survivor curve, such as Fig. 5, curve 3, could yield a concave graph if the drawing out of the original

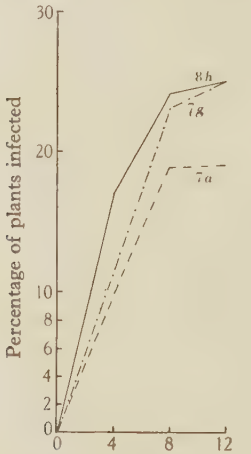

Fig. 10.

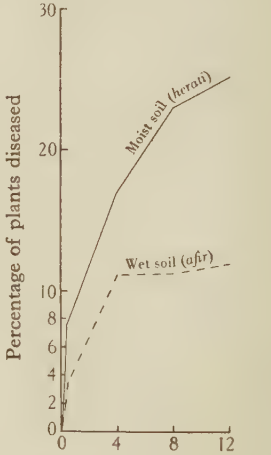

Fig. 11.

Fig. 10. The infection-depth curve in Fig. 9 redrawn in a four-point graph to show possible theoretical infection-depth graphs for flag smut in moist and wet soils.

Fig. 11. Actual disease-depth graphs obtained in the experiments in moist soil (*herati* condition) and wet soil (*afir* condition) for comparison with Fig. 10.

survivor curve were excessively marked. But if the final curve were compounded of two such sigmoid curves on different scales, then the curve and the graph of the lower half would more likely be concave. The concavity of the lower of the flag-smut curve may therefore signify more complexity than occurs with the other diseases, and thus lend some support to the explanation proposed.

Concavity in the curves of the other simpler diseases could also occur if some factor, e.g. temperature, changed during the experiment, giving an irregular sigmoid curve; but this change would need to be great and this is unlikely. None of these other curves is concave, except that for broom corn: this is discussed under soil moisture.

(3) *The influence of soil moisture*

The most noticeable characteristic of the soil-moisture effect is its perfect consistency throughout all diseases and all depths. The difference between the pairs of curves varies, but wet soil always gives less disease than moist soil. Secondly, it is clear that the effect of soil moisture is smaller than that of depth of planting, except perhaps for flag smut. Thirdly,

the effect of moisture increases with depth so that pairs of curves gradually diverge as depth increases. This divergence is small with covered smut of barley and grain smut of millet, it is greater with flag smut, greater still with bunt, where the curves turn in opposite directions after 8 cm. depth, and most pronounced of all in grain smut of broom corn, where the direction of the curves begins to change very early after only 4 cm. depth. The reduced disease in wet soils cannot be due in these experiments either to temperature or to a longer susceptible period which tend rather to minimize the real effect of the difference in moisture content. High soil moisture has often been found to be unfavourable to smut diseases, though few workers have experimented with the maximal soil moisture which commonly occurs under the artificial conditions of irrigation. The depressing effect of high soil moistures was observed by Reed & Faris (1924) and Melchers (1933) for grain smut of millet, while Melchers & Hansing (1938) stated that moisture exceeding 28% discourages infection. Faris (1934) considered that high soil moisture tends to be unfavourable, although he made no observations beyond 70% moisture, but that the effect is closely linked with other disturbing factors, such as soil acidity and temperature. Miller & Millikan (1934) concluded that high soil moisture tends to reduce flag smut of wheat.

The inhibitory action of high soil moisture is generally assumed to be caused by poor aeration. This suggestion does not yet appear to have been proved for smut diseases, but if correct, it is probable that the effect would increase with depth, thus producing the kind of gradually diverging pairs of curves shown by covered smut of barley or grain smut of millet (Figs. 1, 2), which may be taken as the normal case.

In flag smut the soil-moisture effect is unusually large, but this may be connected with soil-moisture conditions before sowing time influencing the germination of soil-borne spores and increasing disease. The abnormally large effect of soil moisture in flag smut may then be due either to inhibition of disease by lack of aeration in wet soil, or to active encouragement of disease in moist soils by pre-soaking of soil-borne spores, or to both factors operating at the same time in opposite directions.

The cases of bunt of wheat and grain smut of broom corn are interesting, for the soil-moisture effect apparently becomes so great in deep sowings that it far exceeds the effect of depth. Perhaps poor aeration may operate more severely on one disease than on another, and aeration conditions may soon become so extreme that infection in wet soil can scarcely take place at 12 cm. depth: the decreases in the curves would thus simply represent extreme cases of poor aeration.

However, this explanation would seem to be in contradiction with the results for grain smut in millet and in broom corn (Fig. 2). Here, only one causative fungus is concerned, and it is unlikely that the fungus should be only slightly inhibited in development when growing in the presence of millet planted at 12 cm. depth, and almost completely inhibited when growing under the same conditions in the presence of broom corn. It is not possible to be certain, for the evidence is that of disease, and it is possible that the reaction of a different host with regard to aeration is not the same.

(4) *A suggestion of destructive parasitism under extreme conditions*

A more likely explanation of the reduction of disease with depth in the cases just mentioned may be that diseased plants have failed to appear because they have been killed in the early stages of infection. Fewer seedlings appear as depth of planting increases, an effect

especially marked in wet soils. Seedlings which reach the surface are at first long and etiolated and sometimes show marked abnormality in colour, breadth, length and strength of leaves, as noticed in the experiments with flag smut. It is possible that if such already abnormal plants become infected with a smut disease, the total effect may be sufficient to cause death. The infected seedlings would die early, leaving a higher proportion of healthy seedlings to grow and ultimately produce a relatively healthier crop. Some support for this suggestion appears if we consider the varying behaviour of the four parasites towards their five hosts.

The most difficult case to explain is the inconsistent behaviour of *Sphacelotheca Sorghi* on millet and on broom corn. This fungus has a wide range of effect upon different varieties of its host. Of some varieties of millet in India Butler (1918) states that scarcely a sign of the disease can be seen externally even at harvest; the shape and size of the grains are unaltered, no elongated spore sac is produced, and a slight reddish colour on the envelope is the only external sign of disease: host and parasite live together harmoniously without apparent harm until just before harvest. On Egyptian millet the fungus has obvious effects on its host at harvest time when the long spore sacs are produced, the stamens sometimes being affected; it is clear that the parasite becomes harmful earlier. On broom corn, however, infected plants are shorter than normal, and although only a few grains in each head are attacked the spore sacs are obvious: here the fungus harms the host earlier, before the heads appear.

Possibly this harmful influence might operate still earlier if infection occurred on already abnormal seedlings planted deeply in wet soil: then the fungus would soon become destructively parasitic and the seedling would die.

The same argument may be applied to bunt to explain the decreased disease in seedlings deeply planted in wet soil. Plants attacked by *Tilletia tritici* are frequently shorter than normal and are often said to bear fewer tillers: these effects are generally reported to be less with *T. laevis*. Sampson & Davies (1927) found no difference in germination of bunt-contaminated and bunt-free seed, but they observed differences of 25–37% in the stand of plants: other effects were sometimes differences in height and dry weight in seedlings, reduction of length of straw at maturity, varied effects in ear length and, perhaps, decrease of root development. These authors worked mainly with *T. tritici*, but they found similar though less marked effects with *T. laevis*. Crepin *et al.* (1937) cited various papers which report the death of seedlings from emergence to earing, one mentioning that 20–25% of plants die in crops from bunt-contaminated seed. The authors themselves considered that, under unfavourable circumstances, including very wet soil, it is not impossible that bunt is a supplementary cause of death, but they were unable to prove it, although recently there is some confirmation in two varieties out of three. Crepin *et al.* consider that the magnitude of the effect of the fungus on the host depends more upon the form rather than the species of the parasite.

The argument should also be extended to flag smut, for in this disease the fungus is obviously harmful, since it often prevents ear formation, affects the leaf area at an early age and not infrequently kills its host while still young. For wet soil there is here no decrease in the curve, but there is a marked flattening which is more significant than with the three other diseases because there are two reasons why, in this case, disease should increase with depth—the usual factor of a lengthened susceptible period, and the unusual factor of the

coleoptile meeting more inoculum as it grows through the spore-infested soil. The curve in moist soil also shows a tendency to flatten, although there are here three reasons—a lengthened susceptible period, increased inoculum and more potent (pre-soaked) inoculum —these together tending to increase disease much more than is observed or expected, for example, in covered smut of barley. Possibly this may explain the difference between the shape of the expected theoretical curve of infection (Fig. 10, curve 7*a*) and the actual curve of disease shown in Fig. 11.

The depth-disease curve for broom corn in moist soil also shows a tendency to flatten, but significance must not be attached to such small differences which could equally well be caused by other factors. On the other hand, there does not appear to be a record of any harmful effect on the host in the case of covered smut of barley, and in the deepest plantings in wet soil there is no sign of a decrease in the curve of disease.

(5) *The depth effect as an instrument and as a problem in research*

The effect of depth of sowing in these five diseases suggests application to others, e.g. seed-borne smuts, such as loose smut of oats, and other seed- and soil-borne, seedling-infecting diseases, such as those of the *Helminthosporium* group. The response of the flower-infecting smuts, where the problem of penetration is different, would be interesting to determine.

It is possible that the depth effect might be used as a tool in studying general problems of seedling infection, as it seems to have the value of separating and varying, within limits, the effect of time, independently of other factors. Porter & Melhus (1932) and Greeves & Muskett (1936) invoke the effect of time to explain why the incidence of seedling rot of water melons and pre-emergence killing of swede seedlings is high at low temperatures and low at high temperatures, although the later phases of these two diseases are severe at high temperatures only. A depth of planting experiment with these two diseases might test the suggestion these investigators have put forward.

In the case of smuts the time effect appears to operate by delaying the end of seedling susceptibility, which is associated with the rupture of the coleoptile and emergence of the green leaves. Thus attention is redirected to the comparatively sudden acquisition of immunity by the seedling about the time that the foliage leaves emerge, a fact long recognized as important in the pathology of smuts. Further study might show whether this is related to the actual rupture of the coleoptile apex which is so closely concerned in the distribution of growth substances, or to the death of the coleoptile, or to the exposure of the coleoptile to light. In the last case the depth effect might be imitated by exposure to light at various stages of growth as Sierp (quoted by Boysen-Jensen (1936)) has shown that the coleoptile of *Avena* can be made to complete its growth at any time between 2 and $4\frac{1}{2}$ days by varying the intensity of light: his graph is reproduced by Boysen-Jensen (1936). On the other hand, this sudden immunity may be related to the occurrence of photosynthesis in the green leaves, or perhaps to that more profound protoplasmic change which needs very little light, but allows the plant to pass from the etiolated to the normal form of growth (Maximov, 1930). At the same time the possibility that the susceptible period may extend beyond the stage of emergence of seedlings must not be overlooked, in view of Faris's (1924*a*) and Tapke's (1938) experiments.

Part III. Application of results to farming practice

(1) *General considerations*

The results presented in the first part of this paper offer the possibility of controlling five smut diseases, principally by regulating the influence of depth of sowing, and to a less extent that of soil moisture at sowing time.

But in general farming practice, depth of sowing is dependent on soil moisture, which is largely uncontrollable, so that it is not possible to plant very near the surface without running the risk of a bad stand, either from weak germination or subsequent drought. Slight modifications may sometimes be possible: thus seeds drilled 4 cm. deep in moist soil in Egypt give a better stand than seeds ploughed in deeply according to the usual *herati* method and will develop only about one-half the amount of disease: again, soaking seeds before planting may often allow shallower planting without greatly increased risk, though this does not generally commend itself to farmers.

In rainless countries, where crops are raised by irrigation, the situation is different because soil moisture, and hence depth of sowing, are easily controllable. Shallow planting in wet soil could be practised as far as is consistent with other farming needs.

(2) *Development of special planting methods under irrigation*

(*a*) *Shallow* afir *sowing.*

At the beginning of this study it was found that *afir* planting gave less disease than *herati*. Comparisons of yield of plots planted in these two ways were undertaken throughout Egypt, and in 1937–8 showed a significantly increased yield of about 7 % in favour of *afir*, the effect being more marked in the Delta (Lower Egypt) than in Upper Egypt where smuts in general are less severe.[1] Such yield comparisons need repetition for several years as the seasonal effect may be considerable. It is clear from the later experiments that further improvement in disease control, and perhaps in yield, is possible by still shallower *afir* sowing than usual. Many farmers could do this, but others would still be obliged by circumstances, such as the rotation used and the weediness or saltiness of their land, to continue planting by the *herati* method.

(*b*) *Mud sowing.*

However, fundamental changes in planting methods are possible. The greatest control of disease could be achieved by planting at zero depth and highest soil moisture, i.e. planting on the surface of recently flooded land where soil moisture is at its maximum owing to the fine soil particles collected there. Two adjacent chequer experiments comparing the amount of flag smut developed, with the ordinary *herati* and *afir* methods, shallow *afir* planting and "mud sowing" both on previously dry soil (*afir* condition) and on previously moist soil (*herati* condition) are set out in Table 1, together with details of cultural operations.

[1] Unpublished results of experiments done by the Botanical and Agronomic Sections of this Ministry.

The figures in columns 5 and 6 show increasing control of disease by the ordinary *afir* and the shallow *afir* methods, while the "mud sowing" shows a high degree of control, amply sufficient for farming practice. This result was obtained both with previously dry and previously moist land, and so could be practised by farmers now accustomed to using either the *afir* or the *herati* method.

TABLE 1

Planting method	Cultural operations	Approx. resultant depth of sowing (cm.)	Soil condition and percentage moisture* at sowing depth and at sowing time	Flag smut (%) Exp. 1	Flag smut (%) Exp. 2
Herati	Seed broadcast on moist soil and ploughed in	8	Moist, 25 %	8·1	8·6
Afir (usual)	Seed broadcast on dry soil, harrowed in with a baulk and irrigated	4	Dry then wet, 8 then 32 %	—	3·2
Afir (modified)	Seed broadcast on dry soil, covered by raking, and irrigated	2·5	Dry then wet, 8 then 32 %	2·4	—
Mud sowing (method 1)	Moist soil ploughed and flooded: seed broadcast on surface 1 hr. later	Nil	Sodden, 41 %	0·2	—
Mud sowing (method 2)	Dry soil flooded: seed broadcast on surface 1 hr. later	Nil	Sodden, 41 %	—	0·08

* Approximate mean percentages of weight of the moist soil.

It would be premature to discuss the agricultural features of "mud sowing", apart from the evidence of losses due to disease, until the method has been widely tested, but there are indications that Method 1 is worth examination. There is the possibility of reducing seed rate in some circumstances, there is indication of increased yield, which is probably only slightly due to disease control, losses due to flag smut being difficult to assess in Egypt (Jones *et al.* 1939). There is also an indication of better quality of grain, which may be related to observed early tillering, consequent on early light exposure, and hence more even maturity of grain; there was no sign of increased tendency to lodge.

(*c*) *Other planting methods.*

There is wide scope for the modification of old, or the development of new, methods of planting designed to prevent losses from smut diseases, in conformity with other farming needs, such as good and safe stand, weed control, yield and even quality of grain. Limited experience indicates that on heavy land in most of Egypt simple "mud sowing" appears to be practicable and satisfactory, but in intermediate land it may perhaps be necessary to treat the seed previously to ensure a good stand. The seed may either be soaked before sowing, or it may be "mud coated" by alternate moistening with water and dusting with fine soil. The increased bulk aids more even broadcasting and adds enough weight to sink the seeds slightly in the mud, and so bring them into capillary contact with it; germination is thus improved and the danger of radicles and tiller roots being unable to penetrate the drying mud surface is avoided.

On very light, quickly drying and hardening land it may be found better and simpler, though slightly less effective in disease control, to resort to a "modified *herati*" method of planting in which untreated seed is first broadcast on the surface of previously ploughed and levelled moist land, then buried about 1 cm. deep by two harrowings with a wooden baulk, and finally irrigated immediately afterwards.

(3) *Special planting methods as an alternative to seed disinfection, or to the use of resistant varieties*

It is clear that suitable modifications in planting methods can exert some degree of control over seed-borne smut diseases, while in irrigation countries new methods might be used to control smuts so efficiently that they would be worthy of ranking with the usual alternatives of seed disinfection or the growing of resistant varieties. The choice of one of these three means of control is not a matter of general principle but of expediency in each case. Ultimately, the practical farmers choose the cheapest and simplest in relation to the extra return in cash obtained by preventing losses from disease.

It is interesting to estimate the relative values of these three methods of avoiding losses from smut diseases under irrigation conditions. First, losses from smut diseases are generally small in relation to losses from rusts and to higher yield or quality which may be expected in a new variety. The plant breeder gives priority in his selection programme to yield, quality and rust resistance, and will often be unable deliberately to add the further complication of resistance to smuts. He will, however, choose the more smut resistant of varieties with otherwise similar properties.

There remains seed disinfection and special planting methods. Grain smut of millet and broom corn can be prevented efficiently by shaking the seeds with sulphur in a jar, a process so simple and cheap that no other control method is needed. A similar process can be used to prevent covered smut of barley (Jones, 1934), and there is probably no need to resort to other methods.

In wheat the position is different. No such harmless and very cheap seed disinfectant is yet available against bunt: the seed must either be steeped, which is troublesome, or dusted with copper or mercury compounds, which seem costly to small farmers, are not easily available, and are poisonous. In flag smut no seed disinfectant yet tested has prevented more than about half the disease in Egypt (Jones & Seif-el-Nasr, 1939). In both cases the best means of control is likely to be special planting methods.

Summary and conclusions

1. The incidence of flag smut and bunt of wheat, covered smut of barley, and grain smut of millet and broom corn was found in Egypt to depend very greatly upon the method of planting.

2. This "method of planting effect" was analysed into a factor of depth of sowing and a factor of soil moisture.

3. The cause of the marked influence of depth of sowing is discussed, with the conclusion

that it is probably due, on the one hand, to lengthening of the susceptible stage of the host by deeper planting, and on the other hand to shortening of this stage by rapid emergence into unfavourable aerial conditions with shallow planting. On this assumption a family of theoretical curves of infection plotted against depth of planting has been constructed, both for the simple case of solely seed-borne smuts and for the complex case of seed and soil inoculum; these curves bear some resemblance to the actual curves of observed disease.

4. The smaller influence of soil moisture is constant, wet soil discouraging disease increasingly with depth, presumably owing to lack of aeration.

5. In exceptional cases, which occur only in deeper plantings in wet soil, the influence of moisture becomes so great that there is apparently a reversal of the influence of depth. It is suggested that under such extreme conditions the balance of host and parasite is disturbed early, so that the fungus becomes destructively parasitic, killing some of the already weak seedlings at an early age and thus preventing a proportion of infected plants from appearing as diseased plants in the crop. The fact that this effect is most marked in diseases which are already under ordinary conditions obviously harmful to the young host plant may perhaps support this suggestion.

6. Variation in the depth of planting may be found a useful instrument in research on seed- or soil-borne seedling infecting diseases, for it seems to enable the effect of time to be separated and varied, within limits, independently of other factors. In smut diseases it seems to operate by delaying the rupture of the coleoptile and the consequent onset of resistance of the seedling.

7. The foregoing results might be applied in farming practice to control smut diseases by planting as shallowly, and on as wet soil, as possible. But the master factor of rainfall will not generally allow much latitude in choice or regulation of either factor.

8. In irrigation countries where soil moisture, and hence depth of sowing, are both easily controllable, these results can be applied fully by sowing on the surface of soaked soil. In Egypt this "mud-sowing method" controls flag smut so efficiently that it offers an alternative means of disease control as efficient, or more efficient, than the usual means of seed disinfection or resistant varieties. Under similar conditions it is clear that there is wide scope for evolving and testing special new planting methods designed to insure against disease loss and accord with the other farming needs.

9. The probable relative practical values and positions of these three possible means of control—special planting methods, seed disinfection and the selection of resistant varieties—are assessed.

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REFERENCES

Appel, O. (1924). Das Auftreten und die Bekämpfung des Steinbrandes. *Mitt. dtsch. Ges. Beil. Flugbl.* 16, 1913. (Quoted from Woolman & Humphrey.) Summary of literature on bunt or stinking smut of wheat. *Bull. U.S. Dep. Agric.* no. 1210.

Appel, O. & Gassner, G. (1907). Der Shädliche Einfluss zu hohen Keimungstemperaturen auf die spätere Entwicklung von Getreidepflanzen. *Mitt. biol. Anst (Reichsanst.), Berl.*, **4**.

Boysen-Jensen, P. (1936). *Growth Hormones in Plants*, Eng. trans. 1st ed. New York.

Brefeld, O. (1905). Die Brandpilze. *Unters. Mykol.* Heft xiii, 58.

Butler, E. J. (1918). *Fungi and Disease in Plants.* Calcutta.

Crepin, Ch., Bustarret, J. & Chevalier, R. (1937). Le problème de la création de blés resistant à la Carie. *Ann. Épiphyt. Phytogénét.*, N.S. **3**, 323.

Faris, James A. (1924*a*). Factors influencing infection of *Hordeum Sativum* by *Ustilago Hordei. Amer. J. Bot.* **11**, 189.

—— (1924*b*). Physiological specialization of *Ustilago Hordei. Phytopathology*, **14**, 537.

—— (1934). Factors influencing the infection of wheat by *Tilletia tritici* and *Tillitia laevis. Mycologia*, **16**, 259.

Flor, H. H. (1933). Studies of physiological specialization in *Tilletia tritici* and *T. laevis* in the Pacific North-West. *J. agric. Res.* **47**, no. 4.

Greeves, T. N. & Muskett, A. E. (1936). A temperature study of *Pythium* attack on swede seedlings. *Ann. appl. Biol.* **23**, 264.

Jones, G. Howard (1934). Control of barley diseases. I. Closed smut. *Tech. Bull. Minist. Agric. Cairo*, no. 142.

Jones, G. Howard & Seif-el-Nasr, Abd el Ghani (1939). Control of smut diseases in Egypt with special reference to sowing depth and soil moisture. *Tech. Bull. Minist. Agric. Cairo* (in the Press).

Kulkarni, G. S. (1918). Smuts of Jowar (*Sorghum*) in the Bombay Presidency. *Bull. agric. Res. Inst., Pusa*, no. 78.

Maximov, N. A. (1930). *A Textbook of Plant Physiology*, Eng. trans. 1st ed., p. 303. New York.

Melchers, L. E. (1933). Belated development of kernel smut (*Sphacelotheca Sorghi*) in apparently healthy *Sorghum* plants. *J. agric. Res.* **47**, 343.

Melchers, L. E. & Hansing, E. D. (1938). The influence of environmental conditions at planting on *Sorghum* kernel smut infection. *Amer. J. Bot.* **25**, 17.

Miller, W. B. & Millikan, C. R. (1934). Investigations on flag smut of wheat caused by *Urocystis tritici* Koern. *J. Dep. Agric. Vict.* **32**, 365–80, 418–32.

Moore, M. B. & Allison, C. C. (1935). The distribution of intermediate types of barley smut. *Phytopathology*, **25**, 28.

Morwood, R. B. (1931). Flag smut in wheat experiments, 1931. *Qd agric. J.* **35**, 363.

Noble, R. J. (1923). Studies of *Urocystis Tritici* Koern., the organism causing flag smut of wheat. *Phytopathology*, **13**, 127.

—— (1924). Studies on the parasitism of *Urocystis tritici* Koern., the organism causing flag smut of wheat. *J. agric. Res.* **27**, 451.

—— (1934). Notes on the longevity of spores of the fungus *Urocystis tritici* Koern. *J. roy. Soc. N.S.W.* **67**, 304.

Porter, D. R. & Melhus, I. E. (1932). The pathogenicity of *Fusarium niveum* and the development of wilt resistant strains of *Citrullus vulgaris. Res. Bull. Ia agric. Exp. Sta.* no. 149, p. 123.

Reed, G. M. & Faris, James A. (1924). Influence of environmental factors on the infection of *Sorghums* and oats by smuts. I. Experiments with covered and loose kernel smuts of *Sorghum. Amer. J. Bot.* **11**, 519.

Ruttle, Mabel L. (1934). Studies on barley smuts and on loose smut of wheat. *Tech. Bull. N.Y. agric. Exp. Sta.* no. 221.

Sampson, K. & Davies, D. Walters (1927). The influence of *Tilletia tritici* (Bjerk.) Wint. and *Tilletia laevis* Kuhn. on the growth of certain wheat varieties. *Ann. appl. Biol.* **24**, 83.

Sartoris, George B. (1924). Studies in the life history and physiology of certain smuts. *Amer. J. Bot.* **11**, 617.

Schellenberg, H. C. (1924). *Die Brandpilze der Schweiz*, 1911. (Quoted from Woolman & Humphrey.) Summary of literature on bunt, or stinking smut of wheat. *Bull. U.S. Dep. Agric.* no. 1210.

TAPKE, V. F. (1935). A study of the cause of variability in response of barley loose smut to control through seed treatment with surface disinfectants. *J. agric. Res.* **51**, 491.

—— (1938). Influence of environment, after seedling emergence, on covered smut in barley. *Phytopathology*, **28**, 370.

TAYLOR, J. W. & ZEHNER, M. G. (1931). Effect of depth of seedling on the occurrence of covered and loose smut in winter barley. *J. Amer. Soc. Agron.* **23**, 132.

TISDALE, W. H. (1923). An effective method of inoculating barley with covered smut. *Phytopathology*, **13**, 551.

TISDALE, W. H. & TAPKE, V. F. (1924). Infection of barley by *Ustilago nuda* through seed inoculation. *J. agric. Res.* **29**, 363.

WOOLMAN, H. M. (1930). Infection phenomena and host reactions caused by *Tilletia tritici* in susceptible and non-susceptible varieties of wheat. *Phytopathology*, **20**, 637.

WOOLMAN, H. M. & HUMPHREY, H. B. (1924). Summary of literature on bunt, or stinking smut of wheat. *Bull. U.S. Dep. Agric.* no. 1210.

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THE INITIATION OF INFECTION BY BUNT OF WHEAT (*TILLETIA CARIES*)

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(With Plate 1 and 3 Text-figures)

Introduction

The physiology and cytology of *Tilletia caries* (= *T. tritici*) have been much studied but practically nothing is known of the way in which it gains entrance into the wheat plant. Flor (1932) and Hanna & Popp (1934) showed that it is heterothallic, but neither observed hyphal or sporidial fusions, nor did they show how penetration is accomplished. Kühn (1859) states that the hyphae of *T. caries* pass readily through the host cell walls and become established as intra- and intercellular mycelium. Lang (1917) found that the mycelium was entirely intercellular. Woolman (1930), who was not able to observe penetration, found that the hyphae are intra- and intercellular in the coleoptiles and leaf-sheaths, but that in the main axis they appear to be entirely intercellular. Dastur (1921) figures infection hyphae between the epidermal cells; he states that occasionally the penetrating hyphae pass directly through the cell wall into the lumen of the cell.

It is generally agreed that the single diploid nucleus in the chlamydospore of *T. caries* gives rise, by several divisions, to haploid nuclei which pass through the promycelium one into each of the primary sterigmata. (The nomenclature of the spore forms of *T. caries* suggested by Buller (1933, p. 239) is used in this paper.) There is not the same general agreement about the nuclear condition of the sickle-shaped spores (primary and secondary basidiospores). Dastur (1921) considers that these are uninucleate and result from the fusion of associated nuclei, i.e. they are diplonts. Kniep (1929) maintains that they are binucleate. Paravicini (1917), Flor (1932) and Hanna & Popp (1934) state that they are uninucleate and are haplonts. Buller (1933) suggested that the fused primary sterigmata give rise to binucleate primary basidiospores from which are derived haploid secondary basidiospores. This would be in accordance with the findings of Boss (1927). A similar type of short nuclear association in other smut fungi has been noted by Bauch (1923) and Dickinson (1927*a*). Dastur (1921) found that the infecting hyphae were uninucleate or multinucleate but not consistently binucleate.

The object of these investigations was to study the mode of penetration and the associated cytological happenings.

Materials and methods

The bunt inoculum was obtained from infected heads of Joss wheat grown in the previous season: for comparative studies two varieties were chiefly used; Joss (susceptible) and Hussar (resistant).

The bunt balls were surface-sterilized by dipping in 95 % alcohol and flaming. The contents of the ball were dusted over the surface of freshly solidified non-nutrient agar (1·7 %) and incubated at 18° C. After about 4 days under these conditions, when secondary basidiospores were produced abundantly, slabs of agar about $\frac{3}{4} \times 2$ in. were cut from the dishes and placed face upwards on micro-

scope slides. Two slides were placed in sterilized soil with the agar surfaces facing each other and separated by a space of about 2 mm. Wheat grains which had previously been surface-sterilized ($HgCl_2$, 1 in 500 for 2 min.) were placed so that, on germination, the coleoptiles would grow in the space between the fungus-covered surfaces of the agar. A third slide was placed on top of the other two to prevent contamination of the agar surfaces by the soil above it (Pl. 1, fig. 6). In this way a heavy infection by a pure culture of *T. caries* was ensured; both the dorsal and ventral surfaces of the coleoptile were infected over their entire length. Furthermore, the plants could be removed and fixed without previously washing off the soil particles, a procedure which usually results in the loss of most of the fungus material. The method has other advantages, e.g. the coleoptiles, under these conditions, grow straight and are subsequently more easily dissected, but there are certain technical difficulties which demand particular care in handling the material, e.g. the fungus, unless attached to the coleoptile, is rather easily removed in fixing, washing and staining. Moreover, it is difficult to strip any quantity of the epidermis from the coleoptile without using macerating solutions which, in turn, remove the mycelium or render it unsuitable for staining. This made it necessary to examine the superficial mycelium through tissue about 6 cells in thickness.

The pots were incubated under controlled temperature conditions at 15° C. At certain critical periods some of the pots in the controlled room were removed and subjected to higher and lower temperatures. At times varying from 2–6 days plants were removed from the pots, fixed for 16 hr. in Belling's modified solution and stained in hot cotton-blue in lactophenol (0·1 %) for $3\frac{1}{2}$ min. The time of staining and the strength of the stain were varied according to the feature to be demonstrated. Shorter exposures to weaker cotton-blue were best to show the cytological condition of the superficial mycelium. On the other hand, staining for 1 hr. in a stronger cotton-blue solution at 38° C. and then destaining in lactophenol demonstrated the vacuolated appressorium and mycelium just after penetration. The coleoptile was opened along one side and, after the enclosed leaves had been removed, was mounted in lactic acid with the outer epidermis upwards.

In addition some coleoptiles were embedded in wax and median longitudinal sections were cut at thicknesses of 10 μ, mounted and stained. The most satisfactory results were obtained by staining with carbol-thionin with a counterstain of orange G in clove oil.

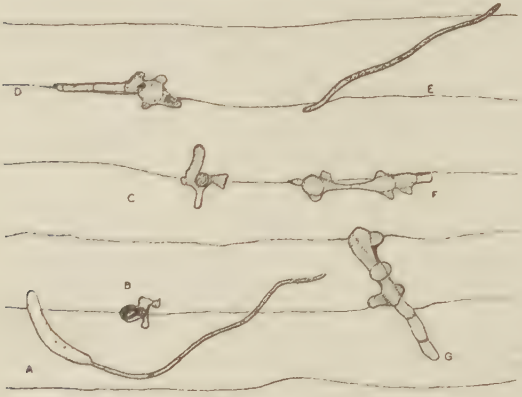
Single spore cultures of secondary basidiospores were established and used for inoculations.

Experimental results

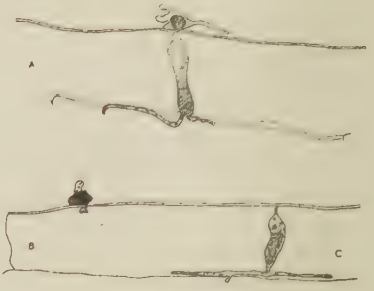
The surface of the coleoptiles was covered with a mat of mycelium. Two types of mycelium were evident: one, derived from the germinating secondary basidiospores, was characteristically narrow (approx. 1·5 μ diam.), non-septate, regular in outline, and occasionally branched; the other showed irregular growth, was greater in diameter (approx. 3 μ diam.) and was more deeply stained (Text-fig. 1 A, B, C, E; Pl. 1, fig. 1). The thick mycelium arose as the result of fusion between two of the narrow, less deeply stained hyphae derived from the secondary basidiospores. The narrow hyphae contained several haploid nuclei which presumably were derived from the single haploid nucleus of a secondary basidiospore.

Upon fusion there was a rapid streaming of contents of both hyphae into the newly formed "fusion hypha" leaving behind completely vacuolated shells which easily became detached. As the protoplasm streamed forward in a hypha septa were laid down behind it. Compatible nuclei became associated in pairs in the newly formed mycelium. Because of the rapidity with which fusion was completed, and as the vacuolated hypha was fragile, "fusion hyphae" with attached secondary basidiospores were not easily demonstrated. Text-fig. 2 A, and Pl. 1, fig. 2 show the formation of a "fusion hypha" after the fusion of germ tubes arising from two secondary basidiospores. It is common to find the haploid hyphae still attached to the darkly stained mycelium especially when there was a tip-to-tip fusion of the germ tubes (Text-fig. 2 B, C). As the vacuolated hyphae became detached close to the "fusion hypha", tip-to-side fusions were less commonly seen.

A striking feature of hyphal fusion was the rapidity with which it took place. By examining material removed from the greenhouse at different periods it could be shown that, under certain conditions, hyphal fusion, the formation of the irregular deeply stained mycelium and the vacuolation of the sporidia were completed within 75 min. On living material observed under the microscope the formation of the "fusion hyphae" was completed within



Text-fig. 1.



Text-fig. 3.

Text-fig. 2.

Text-figs. 1–3. Camera lucida drawings

Fig. 1. Surface of coleoptile. A, E, narrow mycelium; B, C, thick, irregular, deeply stained mycelium; D, F, G, vacuolated mycelium and appressoria.

Fig. 2. "Fusion hyphae." A, fusion of germ tubes arising from secondary basidiospores; B, C, "fusion hyphae" arising from fused haploid hyphae from which basidiospores have become detached.

Fig. 3. Median longitudinal section of coleoptile. A, B, appressoria; A, B, C, swollen subcuticular vesicle and narrow intercellular mycelium arising from it.

45 min. but it could not be determined just when the sporidia became completely vacuolated. It appears that the necessary environmental conditions are exacting but once these are obtained, fusion occurs rapidly. Temperatures of about 13–15° C. favour hyphal fusions especially if, instead of remaining constant, the temperature fluctuates a few degrees on either side of this mean. This, together with the fact that there are limits of humidity outside which fusions will not occur, suggests that the rate of evaporation may be an important factor. Experiments were made to induce hyphal fusions on artificial media with

different *p*H values, C/N ratios and humidities. These were not successful and it is suggested that some other stimulus, possibly supplied by the surface of the host cell, is necessary to induce fusions.

In at least three cases sickle-shaped basidiospores themselves appeared to give rise to irregular, deeply stained mycelia without any apparent fusion, but it could not be determined whether these were primary or secondary basidiospores. Unfortunately, the nuclear condition of this mycelium could not be established. No fusion occurred between the hyphae of a single-spore culture derived from a secondary basidiospore.

The "fusion hyphae" on the surface of the coleoptiles varied greatly in shape and size (Pl. 1, fig. 1), and were not orientated in any particular way. With the appropriate stimulus, an appressorium was formed from below the hypha as a swollen cell which became fastened to the host between the epidermal cells. It is not known what stimulates the fungus to form an appressorium but this is always found at the slight depressions on the surface where the epidermal cells meet.

It was not possible to demonstrate any mucilaginous sheath by using Indian ink or gentian violet. In cases where the appressorium was formed soon after hyphal fusion it assumed a typically heaped, hemispherical shape, with a number of irregular outgrowths arising from it (Pl. 1, fig. 4, and Text-fig. 3 A, B). Failing suitable stimulatory conditions, the "fusion hyphae" continue to develop irregular outgrowths. However, the hyphae rarely grow more than about 35μ before these conditions are attained. Very occasionally, two appressoria were formed in the same mycelium. As the protoplasm streamed into the swollen cell it left behind a septate vacuolated mycelium which remained attached to the coleoptile (Pl. 1, fig. 3; Text-fig. 1 D, F, G).

Penetration was accomplished by means of a small peg through the plant cuticle between the epidermal cells: in every case examined it was intercellular. In section, it is sometimes difficult to determine whether penetration is inter- or intracellular. This is especially the case for hyphae in the host tissues since in tangential section intercellular hyphae often appear intracellular. Examination of the hyphae in cotton-blue preparations of the coleoptile where the mycelium could be traced to a depth of six cells, left no doubt as to their intercellular position. In this method, however, many cytological details cannot be seen which may be observed in well-stained sections. It is necessary to combine both methods to get the complete picture. Several cases were observed where haploid mycelia passed through stomata but no further development occurred and it appeared that this was only a chance happening. Furthermore, no penetration was observed by the promycelium of the chlamydospore or by mycelia derived from fused primary sterigmata or from single secondary basidiospores. It seems then, that, in *T. caries*, hyphal fusions are prerequisite to penetration.

Immediately after penetration the hypha broadened considerably to form a swollen, irregular cell into which passed the contents of the appressorium (Pl. 1, fig. 5; Text-fig. 3 A, C, D). This cell contained pairs of nuclei derived from those which had become associated at hyphal fusion. The swelling takes place only in the plane of the median longitudinal section of the coleoptile, the hypha being otherwise constricted in its growth by the walls of the epidermis between which it grows. In shape it might be compared to a leaf, pressed between pages of a book. It differs from the substomatal vesicles of the rusts which are not restricted in their growth by cell walls more in one direction than another.

This enlarged hypha extended only to the depth of one epidermal cell where it bifurcated to form a typically narrow, somewhat knarled, smut mycelium which was always intercellular. Furthermore, the nuclei, which were in pairs in the subcuticular vesicle, were no longer associated in this narrow intercellular mycelium.

Several varieties of wheat known to be resistant to bunt in the field were inoculated, and, as with the susceptible varieties, hyphal fusion occurred on the coleoptiles followed by penetration.

Discussion

In most of the smut fungi where penetrating hyphae have been demonstrated, evidence as to the origin of the germ tubes is lacking or unconvincing. In at least two smuts the infection hypha has been shown to originate from the chlamydospore (*Ustilago zeae* (Walter, 1934), and *U. avenae* (Kolk, 1930; Western, 1936)) but, until now, no one has observed what happens to *Tilletia caries* immediately prior to penetration.

The heterothallic nature of some smuts and the occurrence of hyphal fusions on artificial media have been used as evidence in presuming that fusions between hyphae occur in nature as a prerequisite to penetration especially where it takes place through the coleoptile. Cytological evidence for this presumption is lacking. It has been shown in *Ustilago zeae* (Brefeld, 1895; Hanna, 1929) that penetration by hyphae from single sporidia is the rule and also that hyphal fusions occur within the host plant. Western (1937) injected a mixture of two monosporidial lines of unlike sex of *U. avenae* between the glumes of oat seeds in nutrient media and distilled water. The hyphal fusions that developed could not be distinguished from those which occurred in artificial cultures but here, as in previous work (Western, 1936), he did not determine where the penetrating hypha had its origin. The present work indicates that in *Tilletia caries*, hyphal fusion is necessary before penetration occurs. This may be true for other smuts also, as Dickinson suggests (1927*b*). At the Conversazione of the Royal Society of London, May 1927, he demonstrated preparations of *Ustilago Kolleri* showing penetrating mycelia arising from fused hyphae, but this observation unfortunately has not been published.[1] While it appears that, in *Tilletia caries*, hyphal fusions are prerequisite to penetration, it is possible that hyphae derived from fused primary sterigmata, primary basidiospores or from the chlamydospores might also penetrate the coleoptile, if nuclear association is the only essential for penetration.

It has been accepted generally that cell fusions in the smuts give rise to the dicaryotic condition of the internal mycelium which persists until they fuse in the chlamydospore, but it has not been known, hitherto, where the dicaryophase originated. The present work shows that, at least in *T. caries*, the nuclei become associated by the fusion of hyphae of compatible lines derived from secondary basidiospores prior to penetration. However, the association is comparatively short-lived and the dissociation of the pairs of nuclei once they have passed from the subcuticular vesicle, departs from the conventional idea of a dicaryotic internal mycelium. As only the intercellular mycelium of the coleoptile was studied, it is not known when the nuclei become associated again before chlamydospore formation.

There are three stages during the life history of *T. caries* when nuclei are closely associated:

(1) Following the fusion of primary sterigmata resulting in the formation of a binucleate primary basidiospore (Buller, 1933). This association appears to be loose; no conjugate

[1] Personal communication.

divisions occur, the nuclei separate and pass into hyphae which give rise to numerous haploid secondary basidiospores.

(2) Following the fusion of hyphae derived from secondary basidiospores. Here the association is closer; conjugate divisions occur during the increased protoplasmic activity accompanying the formation of the appressorium and penetration. Once this has been accomplished the contents of the appressorium with the many paired nuclei pass into the subcuticular vesicle.

(3) Immediately prior to chlamydospore formation.

The nuclear condition of the intercellular mycelium in the smuts is not well understood. Some investigators consider that it is binucleate but most report a multinucleate or uninucleate condition. Thus, Dastur (1921) found in *T. caries* that the hyphae in the early stages of infection were not consistently binucleate, and Kolk (1930) claims that in *Ustilago avenae* the number of nuclei in the mycelium varies; some parts were uninucleate, others binucleate, and still others multinucleate. The writer found it impossible to distinguish septa in the deeply stained mutinucleate portions of the mycelium of *Tilletia caries*. Cross-walls were evident, however, in the vacuolated portions which, in those smuts which are systemic, are left behind as shells as the fungus hyphae grow forward in the plant. For all practical purposes, the mycelium of *T. caries* is a non-septate coenocyte. Other smuts may behave in the same way and this could account for the diversity of opinion concerning the nuclear condition of the mycelium. While most of the nuclei would be included in the forward portions of the mycelium, one, two or more may be included between the septa which are laid down as it becomes vacuolated.

Summary

1. The mode of penetration of the coleoptiles of wheat by *Tilletia caries* was studied. A special method was adopted in inoculating the coleoptiles to ensure an abundance of mycelium for examination.

2. Cultures from single as well as masses of secondary basidiospores and from chlamydospores were used as inoculum.

3. Two distinct types of superficial mycelium were seen on the coleoptile. One, derived from germinating secondary basidiospores was narrow, not deeply stained, regular in outline and occasionally branched. The second, resulting from the fusion of two of the narrow type of hyphae, was shorter, irregular in outline and more deeply stained. In it the nuclei were associated in pairs. No fusions occurred between the hyphae of single spore cultures derived from a secondary basidiospore so that in plants inoculated with these cultures "fusion hyphae" were absent.

4. Attempts to bring about hyphal fusion of compatible strains on artificial media of various kinds were unsuccessful.

5. An appressorium formed below the "fusion hypha". Where this came in contact with the plant cuticle between the epidermal cells a small peg developed and penetration was completed. Penetration was always intercellular.

6. Immediately after penetration there was formed an irregular, swollen fungus cell into which passed the contents of the appressorium. In it the nuclei were associated in pairs.

7. At the first cross-wall this swollen cell bifurcated to form narrow, intercellular mycelium in which the nuclei were no longer associated.

I wish to thank Prof. F. T. Brooks and Dr S. Dickinson for their critical suggestions and helpful guidance.

REFERENCES

Bauch, R. (1923). Über *Ustilago longissima* und ihre Varietät *macrospora*. *Z. Bot.* **15**, 241–79.

Boss, G. (1927). Beiträge zur Zytologie der Ustilageen. *Planta*, **3**, 597–627.

Brefeld, O. (1895). *Untersuchungen aus dem Gesammtgebiete der Mykologie. Der Brandkrankheiten des Getreides*, Heft 11. Munster.

Buller, A. H. R. (1933). *Researches on Fungi*, **5**, 207–78. London: Longmans.

Dastur, J. F. (1921). Cytology of *Tilletia tritici* (Bjerk.) Winter. *Ann. Bot., Lond.*, **35**, 399–407.

Dickinson, S. (1927*a*). Experiments on the physiology and genetics of the smut fungi. Hyphal fusion. *Proc. roy. Soc.* B, **101**, 126–35.

—— (1927*b*). Experiments on the physiology and genetics of the smut fungi. Seedling infection. *Proc. roy. Soc.* B, **102**, 174–6.

Flor, H. H. (1932). Heterothallism and hybridization in *Tilletia tritici* and *T. levis*. *J. Agric. Res.* **44**, 49–58.

Hanna, W. F. (1929). Studies in the physiology and cytology of *Ustilago zeae* and *Sorosporium reilianum*. *Phytopathology*, **19**, 415–42.

Hanna, W. F. & Popp, W. (1934). The physiology of fungi causing bunt in wheat. *Proc. 5th Pacific Sci. Congr.* pp. 3195–204.

Kniep, H. (1929). Vererbungserscheinungen bei Pilzen. *Bibliogr. genet.* **5**, 372–478.

Kolk, Laura (1930). Relation of host and pathogen in the oat smut, *Ustilago avenae*. *Bull. Torrey bot. Cl.* **57**, 443–507.

Kühn, J. (1859). *Die Krankheiten der Kulturgewächse; ihre Ursachen und ihre Verhütung*, Aufl. 2, 54–61. Berlin.

Lang, W. (1917). Über die Beeinflussung der Wirtspflanze durch *Tilletia tritici*. *Z. PflKrankh.* **27**, 80–99.

Paravicini, E. (1917). Untersuchungen über das Verhalten der Zellkerne bei der Fortpflanzung der Brandpilze. *Ann. mycol., Berl.*, **15**, 75–8.

Walter, J. M. (1934). The mode of entrance of *Ustilago zeae* into corn. *Phytopathology*, **24**, 1012–20.

Western, J. H. (1936). The biology of oat smuts. IV. The invasion of some susceptible and resistant oat varieties, including Markton, by selected biological species of smut (*Ustilago avenae* (Pers.) Jens. and *Ustilago Kolleri* (Wille)). *Ann. appl. Biol.* **23**, 245–63.

—— (1937). Sexual fusion in *Ustilago avenae* under natural conditions. *Phytopathology*, **27**, 547–53.

Woolman, H. M. (1930). Infection phenomena and host reactions caused by *Tilletia tritici* in susceptible and non-susceptible varieties of wheat. *Phytopathology*, **20**, 637–52.

EXPLANATION OF PLATE 1

Fig. 1. Two types of superficial mycelium on coleoptile; the narrow regular haploid type, and the more deeply stained shorter and irregular "fusion hyphae." ×280 approx.

Fig. 2. "Fusion hypha" formed by fusion of germ tubes arising from secondary basidiospores. One basidiospore is almost completely vacuolated. ×280 approx.

Fig. 3. Vacuolated "fusion hypha" and appressorium attached to surface of coleoptile at the point of penetration. ×600 approx.

Fig. 4. Median longitudinal section of coleoptile through an appressorium showing penetration. Note the beginnings of a swollen hypha immediately after penetration. ×460 approx.

Fig. 5. Median longitudinal section of coleoptile immediately after penetration. The swollen cell bifurcates at the first inner host cell wall to form narrow intercellular mycelium. ×460 approx.

Fig. 6. Method used for inoculating wheat plants with *Tilletia caries*. The coleoptiles grow between two strips of agar on the surface of which is a pure culture of *T. caries*.

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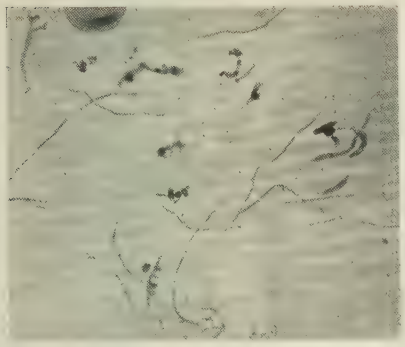

Fig. 1.

Fig. 4.

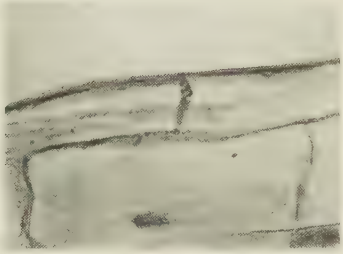

Fig. 5.

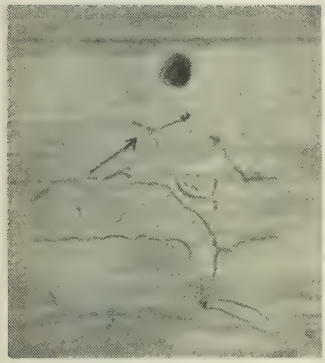

Fig. 2.

Fig. 6.

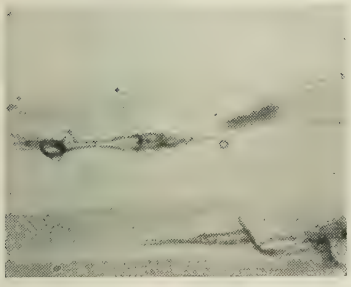

Fig. 3.

CHURCHWARD.—The Initiation of Infection by Bunt of Wheat (pp. 58–64)

SOME NOTES ON A SUSPECTED VARIANT OF *SOLANUM VIRUS 2* (POTATO VIRUS *Y*)

By KENNETH M. SMITH, F.R.S., *Plant Virus Research Station, Cambridge*, and R. W. G. DENNIS, PH.D., *Plant Pathology Station, Corstorphine, Edinburgh*

(With Plate 2)

DURING the last few years routine experiments have been carried out on a field scale to study the natural mode of transfer of *Solanum Virus 1* (potato virus *X*). The experiments involved the use of such plants as tobacco and *Datura Stramonium*, set out in the vicinity of infected potatoes (Smith & McClement, unpublished). It was early discovered that these plants tended to become infected with extraneous viruses and more than one undescribed virus has been isolated in this manner. The purpose of these notes is to describe a virus or virus complex which has definite affinities with *Solanum Virus 2* (potato virus *Y*) and yet differs from it in certain important particulars.

The disease was first observed in the summer of 1935 affecting a tobacco plant, var. White Burley, which was growing near the experimental potato plants but out of contact with them. This is an important point in view of the transmission of *Solanum Virus 1* by means of mechanical contact between healthy and virus-infected plants (Loughnane & Murphy, 1938 · Smith & McClement, unpublished).

The disease was severely necrotic and superficially much resembled that caused by the virus complex *Solanum Viruses 1* and *2* in the tobacco plant (Pl. 2, fig. 1). Indeed, the first tests were made on the assumption that these two viruses were involved in the production of the disease.

EXPERIMENTAL TRANSMISSION OF THE DISEASE

Preliminary inoculation tests early showed that the disease was not a straightforward complex of *Solanum Viruses 1* and *2*. In a series of inoculations to White Burley tobacco, a proportion of the inoculated plants developed the characteristic disease while the rest developed a vein-clearing, followed by vein-banding, a symptom-picture indistinguishable from that caused by *Solanum Virus 2* in the same variety of tobacco. Inoculations from the necrotic disease were then made to a number of solanaceous plants and the interesting fact emerged that it was not possible to produce necrosis on any plant except tobacco. Return inoculations to White Burley tobacco reproduced the necrotic disease except in the case of certain host plants from which only the vein-banding element could be recovered. For example, *Nicotiana glutinosa* was found to be highly susceptible to inoculation with the necrotic disease but reacted as if to infection with *Solanum Virus 2*, showing vein-clearing, vein-banding and slight waving of the leaf margins but no necrosis. Return inoculation to tobacco readily yielded the full necrotic disease which always contains the vein-banding phase as well. The tomato plant behaved similarly but *Lycopersicum racemigerum* yielded only the vein-banding disease on return inoculations to tobacco. When the necrotic phase was once lost in a series of inoculations on tobacco plants it never reappeared.

Neither the full necrotic disease nor the vein-banding phase could be recovered from the following plants, *Schizanthus retussus*, *Solanum nodiflorum* or *Datura Stramonium*. Here the behaviour of the vein-banding virus differs from that of *Solanum Virus 2*, since the latter virus easily infects both *Schizanthus retussus* and *Solanum nodiflorum*. Further details of host range and return infections are given in Table 1.

TABLE 1. *Host range of the necrotic disease, other than the potato plant*

Host plant	Symptoms	Symptoms of return inoculation to tobacco (White Burley)
Nicotiana alata	Bright vein-clearing, veinal mottle and dark green vein-banding; no necrosis	Full necrotic disease
Nicotiana rustica	Very faint mottle, following trace of vein-clearing; no necrosis	Full necrotic disease
Nicotiana langsdorffii	Faint vein-clearing, followed by swelling between veins and faint interveinal mottle; no necrosis	Full necrotic disease
Nicotiana glutinosa	Vein-clearing, vein-banding and waving of leaf margin; no necrosis	Full necrotic disease
Nicotiana noctiflorum	Diffuse vein-banding mottle; no necrosis	Full necrotic disease
Lycopersicum esculentum	Trace of mottle only	Full necrotic disease
Lycopersicum racemigerum	Trace of mottle only	Vein-banding disease only
Solanum nigrum	Vein-banding, mottle; no necrosis	Full necrotic disease
Hyoscyamus niger	Yellow interveinal mottle; no necrosis	Full necrotic disease
Petunia nyctaginiglora	Vein-clearing, bright vein-banding; no necrosis	Full necrotic disease
Salpiglossis variabilis	Narrow vein-banding, small necrotic spots	Vein-banding disease only
Lycium barbarum	Necrotic local lesions	Not recovered
Datura Stramonium	No symptoms	No symptoms

REACTIONS OF THE NECROTIC DISEASE ON THE POTATO PLANT

The disease was transmitted to a number of different potato varieties both by grafting and by sap inoculation using carborundum powder. With a few slight differences the reactions of the potato plants were comparable to those produced by inoculation with *Solanum Virus 2*. It is rather remarkable that from only two potato varieties (President and International Kidney) was it possible to recover the full necrotic disease by reinoculation to tobacco; from all other varieties the vein-banding phase only was recovered.

The results of a series of transmissions to potatoes and return transmissions to tobacco are given in Table 2. It will be seen that the necrotic phase of the disease seems to play little part in the production of symptoms in the potato, all of which appear to be due to the action of the vein-banding phase of the disease.

PHYSICAL PROPERTIES OF THE VIRUS OR VIRUSES CAUSING THE NECROTIC DISEASE

(1) *Thermal inactivation point*

Sap was expressed from tobacco plants showing the full necrotic disease and passed through cheese cloth. About 2 c.c. of this sap were exposed in thin-walled glass tubes to the desired temperature for 10 min. In one experiment no virus was recovered between temperatures of 75–55° C.; at 50° C. however and below, the full necrotic phase developed on the test plants. In a second experiment the vein-banding phase only was recovered at temperatures of 50° C. and below.

TABLE 2. *Reactions of potato varieties to inoculation with the necrotic disease*

Potato variety	Mode and type of infection	Symptoms	Return inoculation to tobacco (White Burley)
President	Tomato graft containing necrotic phase	Small foliar necroses on stock, young leaves with large necroses; leaf-drop streak finally developed	Full necrotic phase
President	Inoculation with necrotic disease using carborundum powder	Veinal necroses followed by lethal leaf-drop streak	Full necrotic phase
President	Inoculation with vein-banding phase using carborundum powder	Diffuse veinal mottle, no necrosis but middle leaves are brittle and drop at a touch	Vein-banding phase
President	Grafted with tobacco containing vein-banding phase	Mottle with grey necroses and light streaking of veins, considerable leaf-drop	Vein-banding phase
King Edward	Tomato graft containing necrotic phase	Diffuse interveinal mottle, middle leaves dead and hanging	Vein-banding phase
King Edward	Inoculation with vein-banding phase using carborundum powder	Slight veinal mottle; lower leaves dropped	Vein-banding phase
King Edward	Grafted with tobacco containing necrotic phase	Veinal mottle, leaf-drop streak	Vein-banding phase
Arran Victory	Tomato graft containing necrotic phase	Faint veinal mottle, no leaf-drop or necrosis	Vein-banding phase
Arran Victory	Inoculation with necrotic phase using carborundum powder	Faint veinal mottle, no necrosis or leaf-drop	Vein-banding phase
Arran Victory	Tomato graft containing vein-banding phase	Faint veinal pallor; lower leaves dead and hanging	Vein-banding phase
Epicure	Tomato graft containing necrotic phase	Necrosis, leaf-drop streak	Vein-banding phase
Epicure	Inoculation with necrotic phase using carborundum powder	Necrosis, leaf-drop streak	Vein-banding phase
Epicure	Tomato graft containing vein-banding phase	Veinal mottle, leaf-drop streak	Vein-banding phase
Epicure	Inoculation with vein-banding phase using carborundum powder	Veinal mottle, leaf-drop streak	Vein-banding phase
Katahdin	Inoculation with necrotic phase using carborundum powder	Veinal mottle, leaf-drop streak	Vein-banding phase
Doon Star	Inoculation with vein-banding phase using carborundum powder	Leaf-drop streak	Vein-banding phase
International Kidney	Tomato graft containing necrotic phase	Leaf-drop streak	Necrotic phase

(2) *Resistance to ageing*

It is in its resistance to ageing that the vein-banding phase differs sharply from *Solanum Virus 2*. It easily retains its infectivity at room temperatures up to 27 days or longer while *Solanum Virus 2* is generally inactivated after about 2 days under similar conditions. The necrotic phase on the other hand appears to be lost after 24 hr. ageing.

(3) *Filtration experiments*

Three experiments on filtration through graded collodion membranes were carried out. The sap was extracted from fully necrotic tobacco plants and clarified by passage through a kieselguhr bed. In the first experiment the full necrotic phase passed both the kieselguhr

bed and the first membrane of A.P.D. 0·584μ. The vein-banding phase alone was recoverable from the next two filtrates through 0·459 and 0·302μ respectively while no virus was recovered from the filtrate of a membrane of A.P.D. 0·110μ. In the second experiment no virus was present either in the kieselguhr filtrate or filtrates from various membranes. In the third test, the necrotic phase passed the kieselguhr bed but only the vein-banding phase passed the membrane of A.P.D. 0·215μ.

These filtration experiments, slight as they are, yet suggest a difference between *Solanum Viruses 1* and *2* and the components of the necrotic disease. In the first place, as already pointed out (Smith, 1932) it is extremely difficult to filter *Solanum Virus 2* through membranes even when of large pore size owing to its high adsorptive capacity, and passage of a mixture of *Solanum Viruses 1* and *2* through a kieselguhr bed usually gives a filtrate containing only *Solanum Virus 1*. In these experiments, however, the full necrotic phase which, as previously stated, always contains the vein-banding phase, passed the kieselguhr bed and afterwards the vein-banding phase passed membranes of four different A.P.D.

This behaviour is the reverse of what occurs in filtration experiments with *Solanum Viruses 1* and *2*; here it is *Solanum Virus 2* which is always left behind while *Solanum Virus 1* easily passes both kieselguhr bed and such coarse membranes.

Dilution end-point

Dilution experiments were carried out with the crude extracted sap of tobacco plants infected with the necrotic disease and the results show a similarity to those obtained under similar conditions with *Solanum Virus 2*. The necrotic phase generally dropped out at dilutions of 1 : 800 though it appeared irregularly even at lower dilutions. The vein-banding phase persisted to dilutions of 1 : 1000 but not beyond that point.

The following protocol gives results typical of the dilution tests:

Dilution	Result on White Burley tobacco
1 : 10	Full necrotic disease
1 : 100	Full necrotic disease
1 : 200	Full necrotic disease
1 : 300	Full necrotic disease
1 : 400	Vein-banding phase only
1 : 500	Vein-banding phase only
1 : 600	Full necrotic disease
1 : 700	Vein-banding phase only
1 : 800	Full necrotic disease
1 : 900	Vein-banding phase only
1 : 1000	Vein-banding phase only
1 : 10000	No symptoms

Resistance to chemicals

(*a*) *Alcohol*

The resistance to alcohol of both components of the necrotic disease is very low, infection was usually lost after exposure for 1 hr. to 50% alcohol though in one such experiment two plants out of six showed the vein-banding phase. Infection with vein-banding was irregular after exposure to alcohol at concentrations as low as 20% while the necrotic phase did not survive exposure to concentrations higher than 10%.

(*b*) *Mercuric bichloride*

The vein-banding phase but not the necrotic element was recovered after exposure for 3 hr. to solutions of mercuric bichloride of 0·05 and 0·005 % concentration.

Insect transmission

(*a*) *Myzus persicae*

White Burley tobacco plants showing the full necrotic disease were colonized with aphides (*Myzus persicae*) which were then transferred to three healthy tobacco plants. The latter developed the typical vein-banding disease but showed no signs of the necrotic phase.

(*b*) *Macrosiphum gei*

Twelve individuals of *Macrosiphum gei* were colonized on a White Burley tobacco plant affected with the necrotic disease. On transfer to healthy plants they induced the vein-banding phase only.

It was not found possible to transfer the full necrotic disease by means of either species of aphis.

Cross-immunity tests

Solanum Virus 3 (virus A). Systemic infection with *Solanum Virus 3* afforded no protection to tobacco plants against invasion by the necrotic disease. This is shown by the following data. On 25 January 1938 six White Burley tobacco plants were inoculated with *Solanum Virus 3*. On 3 February 1938, when systemic symptoms had developed, the plants were reinoculated with sap from a White Burley plant with the necrotic disease. Some weeks later all six plants showed the full necrotic disease.

Solanum Virus 2. The cross-immunity tests with *Solanum Virus 2* were somewhat inconclusive but so far as they go they suggest that no protection is afforded by *Solanum Virus 2* against infection by the necrotic phase. It is hardly feasible to test immunity between the vein-banding phase and *Solanum Virus 2* since the symptoms caused by the two viruses are identical. The following protocols show the kind of result achieved in these tests. Six tobacco plants were inoculated on 25 January 1938 with *Solanum Virus 2*, on 3 February 1938 three of those plants were systemically infected and they were re-inoculated with sap from a tobacco plant with the full necrotic disease. On 30 March 1938 there was no change and no necroses had developed. On 16 November 1938 twelve tobacco plants were inoculated with *Solanum Virus 2* and on 27 November 1938 six plants were chosen showing full symptoms of infection and re-inoculated with the necrotic disease. Three of these plants later developed the typical necrotic symptoms.

In considering these results it is important to remember that the necrotic disease is, in any case, of weak infective power and frequently fails to carry over in a series of straight transfers. It is probably permissible, therefore, to conclude that no cross-immunity exists.

Solanum Virus 1. The necrotic phase does not contain *Solanum Virus 1*; no immunity was afforded in cross-inoculation tests. When *Solanum Virus 1* was mixed with the vein-banding phase virus, a symptom picture developed which was identical with that produced on tobacco by a mixture of *Solanum Viruses 1* and *2*.

Discussion

While these experiments are admittedly incomplete, they seem sufficient to indicate the existence of a distinct variety of *Solanum Virus 2*. The presumed variant resembles *Solanum Virus 2* in its symptoms on tobacco and many other plants but differs slightly in its reaction to *Schizanthus retussus*. It differs very sharply however in its resistance to ageing which is 4 weeks or more at room temperatures compared to about 2 days for the type virus under similar conditions. It also differs in its ability to pass a kieselguhr bed and some of the larger-pored membranes.

Whether the necrotic factor is a separate virus or merely a phase brought about by a particular set of conditions has not been determined.

All attempts to separate the necrotic phase from the vein-banding phase have failed, though it is easy to separate the latter from the former. Furthermore, once this has been accomplished, there seems to be no recurrence of the necrotic phase, but there is some evidence that it can be restored by re-inoculation from a fully necrotic plant.

The only evidence of its existence is the development of necrosis on the tobacco plants and, if it is a separate virus, its properties are all of a negative character. Thus it is less readily filtered than the vein-banding phase, less resistant to dilution, less resistant to chemicals, not transmitted by aphides and is frequently lost altogether in straightforward sap inoculation. It cannot be related to *Solanum Virus 1* since it will not infect *Datura Stramonium* and has none of the physical properties of that virus.

Summary

An account is given of an apparent variant of *Solanum Virus 2* (potato virus *Y*) which differs slightly from the type virus in its symptoms and sharply in its longevity *in vitro*. It is accompanied in the tobacco plant by a necrotic symptom which is suspected to be due to a separate virus of very unstable character.

REFERENCES

Loughnane, J. B. & Murphy, P. A. (1938). *Sci. Proc. R. Dubl. Soc.* **22** (N.S.), 1–15.
Smith, Kenneth M. (1932). *Nature, Lond.*, **130**, no. 3276.
Smith, Kenneth M. & McClement, W. D. (Unpublished).

EXPLANATION OF PLATE 2

Fig. 1. Tobacco plant, var. White Burley, infected with the full necrotic disease.

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SMITH AND DENNIS.—SOME NOTES ON A SUSPECTED VARIANT OF *SOLANUM VIRUS 2* (POTATO VIRUS *Y*) (pp. 65–70)

THE BIOLOGY OF THE CHRYSANTHEMUM MIDGE IN ENGLAND

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(With Plate 3 and 5 Text-figures)

1. Introduction

During the second outbreak of the chrysanthemum midge in England, which started in the latter part of 1936, the question arose as to whether or not parasites were present. In February 1937 a small amount of material was received from Sussex, and parasites, which were kindly identified by Dr Ch. Ferrière as *Eutelus diffinis* Walker, were bred out of the galls. Later, information was required as to the life history of the midge in England, and whether the midge was likely to spread to wild chrysanthemums, such as the ox-eye daisy, and other closely allied plants which occur in the vicinity of chrysanthemum houses. Permission having been given to the writer by the Ministry of Agriculture to keep the midge, investigations on these lines were started in December 1937. The results of the host plant range experiments have been published (Barnes, 1939).

The present paper deals with the biology of the midge in England when studied under two different sets of conditions, namely, inside the laboratory, and in an unheated glasshouse at the Rothamsted Experimental Station, Harpenden. Material with which to start the experiments was received from Mr A. S. Buckhurst from an infestation in South Wales. Midges started emerging from this material, which was kept in the laboratory, on 16 December and continued until 26 February. In all 584 midges emerged. Parasites started emerging on 22 January and continued until 15 March. By this date sixty-two parasites had emerged, giving a relative parasitism of nearly 10%.

2. On Cranford Yellow variety

A. *In the laboratory*

Temperature. The laboratory was chosen as a site for studying the life history on account of the supposedly equable temperature and the rather low humidity.

It was found that the range of temperature was considerably more than one might have supposed, but still less than outside in the unheated glasshouse (see § B). For instance, in January 1938 the maximum was 17° C. (62·6° F.) and the minimum 7° C. (44·6° F.). In February the room was colder, but it became warmer in March. Unfortunately the temperature was not read during April and most of May. By June the maximum had reached 24° C. (75·2° F.) and the minimum was 18° C. (64·4° F.). The temperature remained about the same in July and August, with the exception of one week in August when the maximum reached 25° C. (77° F.). During September–November the room was cooler again. In December there was one period of 3 days in which the maximum was 9° C. (48·2° F.) and the minimum 4·5° C. (40·1° F.). The total range for the period under consideration was from 25° C. (77° F.) to 4·5° C. (40·1° F.).

Plants used. The plants used for breeding the midges, both in the laboratory and in the glasshouse, were all subdivisions of one plant of Cranford Yellow. This was an attempt to get as uniform a medium for growth as possible. It was probably successful as far as different plants of the same variety may

influence the rate of development of the midges, but the seasonal differences in rate of plant growth, availability of sap, and rapidly growing plant tissue far outweigh such considerations. It was comparatively simple to keep the plants in a good state of health during the spring and early summer months; e.g. two plants heavily infested with midge were kept from early January until July when they died just before flowering. During August–January the plants were much more difficult to maintain and comparatively few survived more than 3 months. They did not grow well, became brittle and the basal leaves turned brown. This may have been due to the difficulty of watering them adequately and the extra amount of gas being used on the laboratory bench.

Emergence of adult midges. The exact time of emergence was not observed, but it was obvious that it took place in the early hours of the morning. No male was seen emerging after 9 a.m., and the males were nearly always dead or moribund by this hour. This early death may have been partly due to the dryness of the atmosphere, since in the glasshouse they lived longer. The females, also, mostly had emerged by 9 a.m., but emergence of a few individuals was observed as late as 11 a.m.

Mating. This took place very soon after emergence of the female, but many of the females emerged too late in the day to be fertilized as all the males had died. Some of these tardy midges were, however, fertilized by the males emerging the next day, providing the number of newly emerged females was not too great. There was a definite preference on the part of the males to mate with freshly emerged females.

Longevity of adult midges. The males lived only a few hours, usually less than six. The females, on the other hand, lived longer. The majority of the fertilized females died by the evening of the day on which they emerged, but exceptionally such midges have been observed alive 2, 3 and even 5 days after emergence. Unmated females usually lived 2–3 days.

Oviposition. This commenced within about ½ hr. of mating and continued assiduously throughout the forenoon, was less continuous during the afternoon, and normally was completed by about 4 p.m. Occasionally, however, oviposition was seen throughout the second day and once on the morning of the third day. Egg-laying takes place on the rapidly growing parts of the plant, namely, in the buds, and among the folds of the very young leaves (Pl. 3, fig. 1). The eggs are bright red and just discernible by the naked eye when present in some numbers.

Duration of immature stages. No attempts were made to ascertain the exact duration of the egg, larval or pupal stages, but in the case of the first generation during January 1938 the egg stage lasted about 10 days, in the second generation at the end of March 1938 the larvae had hatched in 6 days.

Appearance of galls. Observations were made in order to discover how soon after oviposition the galls became visible (see Table 1). By "appearance of galls" is meant the first occasion on which definite galls or protuberances were clearly visible without any doubt. It can be seen that during the early months of the year galls were not obvious until 5–7 weeks after oviposition (Pl. 3, fig. 2). In April and May the interval was much shorter, about 2–3 weeks, while in June the period was under a fortnight. In October 3 weeks and in November–December 4 weeks were the intervals necessary for certain recognition of attack by the non-specialist. Much shorter times were adequate for diagnosis by the expert who can see the incipient galls.

Number of generations. The method adopted was to allow a varying number of females to oviposit on plants isolated in separate pots. As soon as the midges derived from these

eggs began to emerge fresh pots were set up in a similar manner. At the same time in some cases the originally infected pots were retained. These latter plants became infested with the eggs of the midges emerging from them. However, all the midges were usually taken out of the cages by 11 a.m., so that only a small fraction of the available eggs could have been laid on the plants. This is particularly so during the winter when oviposition starts later in the day than in the summer. In some cases, however, the plants were destroyed, in others they died, after maintaining one completed generation. In order to facilitate discovery of the midges a thin covering of white sand was placed on the soil in each pot. The daily emergences in a number of pots are set out in Text-fig. 1. There was very little

TABLE 1. *Period in days from oviposition until galls were visible under laboratory conditions*

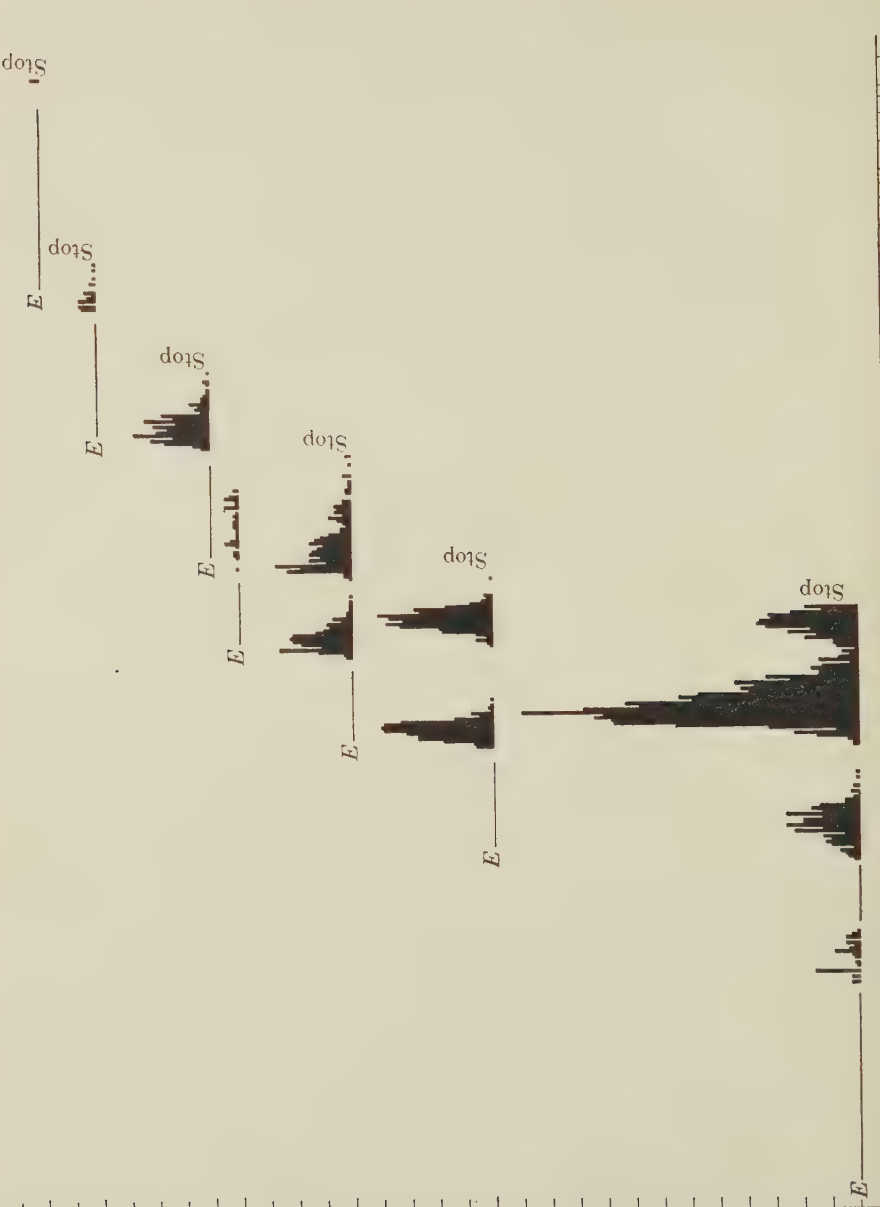
Oviposition	Appearance of galls	Period in days
6 Jan.	14 Feb.	37*
7 ,,	14 ,,	38*
8 ,,	1 Mar.	52
22 Mar.	12 Apr.	21
26 ,,	12 ,,	17
5 May	27 May	22
7 ,,	24 ,,	17
9 ,,	24 ,,	15
9 ,,	27 ,,	18
9 June	22 June	13
9 ,,	22 ,,	13
15 June	3 July	18
15 ,,	30 June	15
17 ,,	3 July	16
1 Oct.	22 Oct.	21
21 Nov.	19 Dec.	28

* Galls just visible; in all other cases obvious.

overlapping of generations until after the third generation on the same plant. The smaller numbers of midges emerging from those plants infected later in the year is largely owing to the poorer general condition of the plants, particularly as regards fresh growth.

In the 12 months following 5 January 1938 there were seven completed generations and the eighth one had been showing galls clearly for about a fortnight. Oviposition started early in January and the adult midges of the first generation first appeared late in March, taking a mean of 77 days. This flight of midges lasted about 20 days. As the season advanced the generations followed more rapidly, the second (March–May) requiring on the average 44 days, the third (May–June) 39, the fourth (June–July) 34 and the fifth (July–August) only 29 days, the shortest time between the date of oviposition and the first appearance of adult midges being 27 days. In the autumn this period lengthened, the sixth generation (August–September) took on the average 43 days, and the seventh (October–November) was again slightly slower, 47 days. The eighth generation (November–February) resembled the first, taking 83 days as compared with 77. Details of these periods in the various pots are shown in Table 2.

Number of midges. The total numbers of midges emerging per month from all plants in



Text-fig. 1. Daily emergences of eight generations of chrysanthemum midges on individual plants January 1938–February 1939 in [illegible] Experimental Station. *E*=dates on which midges started ovipositing.

the laboratory are shown in Table 3, the January and February figures being those of the original stock (see p. 71). The majority of the midges emerged in June and July, notwithstanding the fact that just as many plants were under observation later in the year. This is no doubt partly due to the difficulties experienced in keeping the plants in good growing condition later in the year.

TABLE 2. *Dates of oviposition, first emergence of adult midges and the number of days from oviposition to appearance of the first midge in the different generations under laboratory conditions during* 1938–9

Oviposition	First adult midge	No. of days	Mean	No. of generation
7 Jan.	26 Mar.	78		
5 ,,	30 ,,	84		
5 ,,	23 ,,	77	77	First
8 ,,	26 ,,	77		
6 ,,	22 ,,	75		
15 ,,	27 ,,	71		
26 Mar.	9 May	44	44	Second
22 ,,	5 ,,	44		
9 May	15 June	37		
5 ,,	15 ,,	41	39	Third
7 ,,	15 ,,	39		
9 ,,	17 ,,	39		
16 June	21 July	35		
17 ,,	21 ,,	34	34	Fourth
16 ,,	20 ,,	34		
15 ,,	17 ,,	32		
20 July	18 Aug.	29		
18 ,,	14 ,,	27	29	Fifth
19 ,,	18 ,,	30		
19 Aug.	30 Sept.	42		
20 ,,	30 ,,	41	43	Sixth
25 ,,	10 Oct.	46		
5 Oct.	18 Nov.	44		
1 ,,	19 ,,	49	47	Seventh
2 ,,	19 ,,	48		
20 Nov.	14 Feb.	86		
21 ,,	12 ,,	83	83	Eighth
22 ,,	9 ,,	79		

TABLE 3. *Numbers of midges emerging per month during* 1938 *in the laboratory*

Jan.	558	July	1899
Feb.	20	Aug.	732
Mar.	67	Sept.	964
Apr.	107	Oct.	577
May	437	Nov.	165
June	2613	Dec.	16

Numbers of midges per plant. The numbers of midges a single plant can maintain is large. In one case where three generations were completed and a fourth had started to emerge before the plant finally died, a total of 1905 midges developed, while on another plant the equivalent number was 2049. Table 4 shows the rate of increase starting from eleven and five female midges respectively.

TABLE 4. *Rate of increase during three completed generations on single plants under laboratory conditions*

Parent females	No. of generation: First	Second	Third	Fourth partial	Total
11	19	165	1673	48	1905
5	59	274	1391	325	2049

B. *In an unheated glasshouse*

Temperature. Although the breeding experiments were started in the glasshouse late in March, the temperature was not observed until late in May; since that date a recording thermograph has been in a shaded position on the staging.

From May to the beginning of October both the side and top lights of the glasshouse were kept permanently open. The side windows were kept shut after the beginning of October, and from mid-October to February the top lights were also kept shut except for occasionally airing for a few hours on the milder days. The top ventilator running along the centre of the house was also shut for about a month starting 18 December. During the heat of the summer's day sheets of brown paper were pinned on the inside of the roof to prevent the sun shining directly into the house, and the latter was kept well watered down. The maximum temperature never reached 30° C. (86° F.) except on one occasion in September, when the house was closed for fumigation and it had been omitted to pin up the brown paper sheets, and on one occasion in April 1939. Each light on the west side of the roof was covered during the summer with black muslin to help in keeping down the temperature during the afternoon. In the winter it was changed to white. The side windows always had white muslin coverings.

The range in temperature was considerable both seasonally and weekly. The same is true of the daily range when the thermograph charts are examined. The maximum in June was 27·5° C. (81·5° F.), in July 29° C. (84·2° F.), in August 28·5° C. (83·5° F.), in September the same, omitting the temperature of 30° C. which was reached during fumigation, in October 25° C. (77° F.) and even 24° C. (75·2° F.) in November. In December the temperature never rose more than 15° C. (59° F.), while in January 1939 20° C. (68° F.) was once reached. In February the maximum was 27° C. (80·6° F.), in March 25° C. (77° F.), in April 30° C. (86° F.) and in May 28° C. (82·4° F.). The minimum temperature rose from 5·5° C. (41·9° F.) in June to 7·5° C. (45·5° F.) in July and to 6·5° C. (43·7° F.) in August, although there were several weeks during the latter 2 months in which the minimum was about 10–13° C. (50–55·4° F.). In September the minimum fell to 4·5° C. (40·1° F.), while in October and November it went down to freezing-point. In December [−1·5° C. (29·3° F.)] and January [−1° C. (30·2° F.)] it was below freezing. In February, March, April and May the minima were −1, −0·5, 1 and 4° C. respectively. The total range from March 1938 to May 1939 was from 30° C. (86° F.) to −1·5° C. (29·3° F.).

As the individual chrysanthemum plants were enclosed in muslin cages, the temperatures in the immediate proximity of the plants were a degree or so different from these recorded temperatures. In December and January the plants were frosted on several occasions.

Precautions taken to prevent midges escaping. Each light, all the side windows and the ventilators, were covered with muslin to prevent midges from escaping to the exterior. Each plant on which midges were breeding was enclosed in a muslin cage. Finally, plenty of uncovered chrysanthemum plants were kept between the infested plants and the glass sides of the house in order that any midges escaping from the latter plants might be attracted to oviposit before reaching the sides of the house. These plants were examined at frequent intervals, but no sign of infestation was seen.

Plants used. The plants used for breeding the midges both in the glasshouse and in the laboratory were subdivisions of one plant of Cranford Yellow. The plants remained healthy throughout the time of the investigations, except that in late December and January some white mould appeared on the old flowering stems that were dying and on some of the basal leaves of the new young growth. The plants developed flowers in the late summer but no disbudding was done. These flowering shoots withered and died in the autumn but were allowed to remain on the plants until early March 1939. This was done to see for how long midges would continue to emerge from apparently quite dead old flowering tops. Fresh growth appeared at the base of the plants in the early autumn.

Emergence of adult midges. This again took place early in the morning. One factor controlling emergence seems to be light, since on bright mornings during the summer emergence was completed earlier than on bright mornings during the winter. For example, very few emergences were observed after 9 a.m. during the summer months, but in the winter the proportion of midges emerging after 9 a.m. was comparatively high. It appears possible that the time of emergence bears some direct relation to the time of sunrise. In addition, more emergences seemed to take place on bright sunny mornings than on dull ones irrespective of the actual temperature. The threshold temperature for emergence is low. Only on a few days during the winter of 1938–9 did no midges emerge (see Text-fig. 2). A few emergences were observed in December, even when there was frost on the plants and the air temperature was well below freezing.

Activity, mating and oviposition. On bright sunny mornings the activity of the midges is much greater than on dull days. Mating takes place soon after the emergence of the females and oviposition commences almost immediately. It was exceedingly difficult to find virgin females in the cages during the summer at 9 a.m. on bright summer mornings, but on dull ones the males were less active and unfertilized females could be found up to noon. Each male mates with several females if they are available. In dull weather the males are sluggish and the females lay their eggs more slowly. On the other hand, egg-laying is actively in progress by 9 a.m. during bright summer weather and is usually completed by noon. Occasionally it continues during the afternoon. In dull and even bright weather during the winter oviposition continues for up to 3 days, and the same is true in colder as compared with warmer weather. The threshold temperature for mating appears to be slightly higher than that for emergence, since on some days although emergence took place mating did not. The threshold for oviposition seems to be almost the same as that for mating, because, as soon as the females were fertilized, egg-laying nearly always took place.

During the summer midges had a tendency to fly upwards and congregate at the tops of the cages. In the late autumn and winter the tendency, particularly of the females, was to assemble at the base of the cages. This change over can be explained by the fact that during the summer the actively growing parts of the plants were towards the tops of the cages, whereas during the winter the new growth was basal. When mating is completed in the case of the males, and when oviposition is finished in the case of the females, the tendency is to fly towards the source of light.

Duration of the egg stage. During warm weather young larvae hatched 3–4 days after the eggs had been laid, but in the winter the egg stage lasted as long as 2 weeks.

Appearance of the galls. The time which elapsed between egg-laying and the first appearance of the galls is given in Table 5. In late March–May galls were clearly visible after 6–7 weeks. In May–June this period was 4–5 weeks, and in June–July slightly less. Data are not available for later months in the summer and winter on Cranford Yellow variety.

Number of generations. The method adopted was the same as in the laboratory experiments with the exception that, as all the plants remained healthy throughout the year, they were, with three exceptions due to lack of space, retained throughout the investigations. Hence, the first two pots each containing a single plant were infected with five and one female midges respectively in late March and early April 1938. Subsequently, two more pots were infected with ten and six female midges in June, one with nine females in the latter part of July and two further pots with four and six females in late August and early

September. In early November four more pots were started with five, five, nine and seventeen midges respectively.

The daily emergences in a number of pots are set out in Text-fig. 2, which shows that the numbers of midges emerging from day to day are much more variable than under laboratory conditions (see Text-fig. 1). Also, considerable overlapping of generations took place after the second generation: e.g. in the first two pots set up (only one is shown in Text-fig. 2) there were very few days after 19 July 1938 on which no midges emerged. These were chiefly a few very cold days in December and early January. Naturally, this overlapping only occurs to such a degree in those pots which were really heavily infested with midges. It was also found that midges emerged in certain cages months after the infestation had apparently died out.

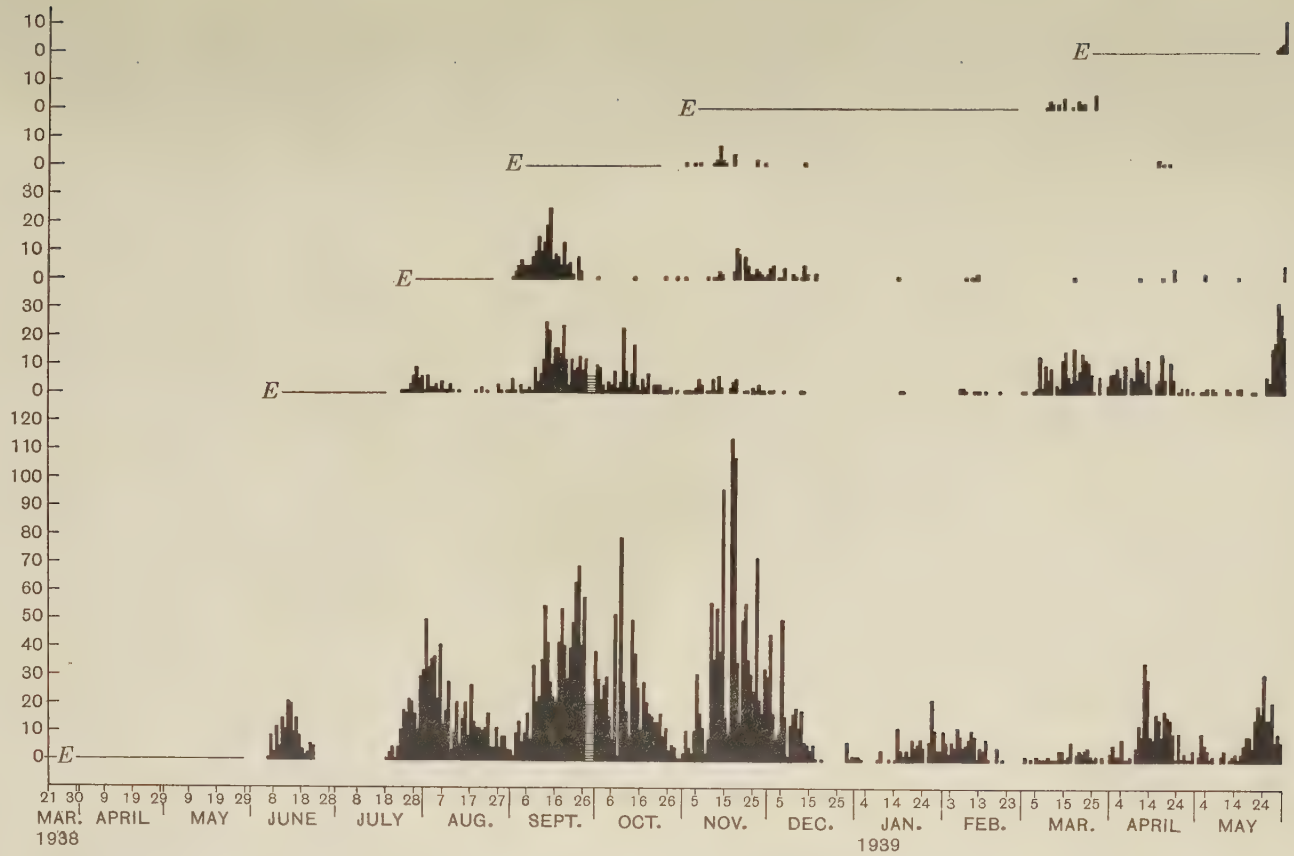
TABLE 5. *Period in days from oviposition until galls were visible under unheated glasshouse conditions*

Oviposition	Appearance of galls	Period in days
27 Mar.	14 May	48
1 Apr.	14 „	43
6 May	8 June	33
10 „	8 „	29
7 June	5 July	28
8 „	5 „	27
8 „	5 „	27
10 „	5 „	25

Commencing with ovipositing females at the end of March 1938, there were four completed generations during the 12 months with a partial fifth one (which had to be bred on September White, Goacher's Single and Dobbie's Brilliant owing to a temporary shortage of Cranford Yellow; see Table 11 entries of oviposition 2–8 November, showing galls in December and early the following January). The first adults derived from eggs laid late in March appeared in early June, the first emergence on the average being 71 days after oviposition. The second generation took about 43 days (June–July) to start emerging, the third took 30 days (August) and the fourth took about 66 days (September–early November). Details of these periods in the various pots are shown in Table 6. Midges of the fifth generation started emerging on an average 136 days (November–March) after oviposition.

Midges continued emerging from apparently dead old flowering shoots up to February 1939. Oviposition took place on the young basal shoots from October onwards.

Effect of fumigation. During late September 1938 the glasshouse was heavily fumigated. On 28 September the house was closed and approximately triple the ordinary dosage of nicotine was used. The muslin cages were not removed from the plants. The glasshouse was not revisited until the morning of 30 September when it was opened up. Considerable numbers of midges, apparently those that had emerged on 28 September, were then found dead at the bottom of the cages; others were found partially emerged and may have been the midges attempting to emerge on 29 September. Finally, there were other midges apparently newly emerged and behaving quite normally, e.g. egg-laying. It is thus evident that, in order to be effective, nicotine fumigation would have to be carried out every day. The total of the midges found alive and dead in each pot on 30 September was divided by 3



Text-fig. 2. Daily emergences of chrysanthemum midges on individual plants, March 1938–May 1939, in unheated glasshouse, Harpenden, Herts. *E* = dates on which the midges started ovipositing. ≡ = the numbers of midges emerging on the day of fumigation and the two subsequent days.

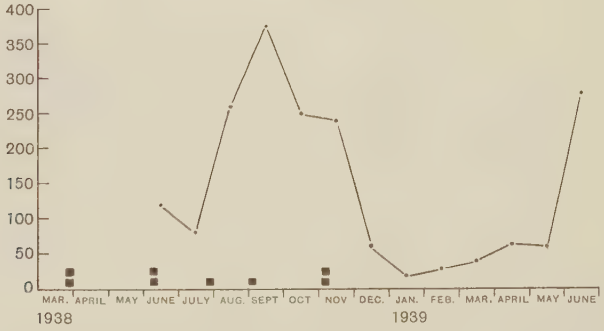
TABLE 6. *Dates of oviposition, first emergence of adult midges and the number of days from oviposition to appearance of the first midge in the different generations under unheated glasshouse conditions during* 1938*

Oviposition	First adult midge	No. of days	Mean	No. of generation
27 Mar.	7 June	72	71	First
1 Apr.	9 ,,	70		
7 June	19 July	42	43	Second
9 ,,	20 ,,	41		
8 ,,	24 ,,	46		
10 ,,	22 ,,	42		
25 July	1 Sept.	38	30	Third
24 ,,	19 Aug.	26		
22 ,,	18 ,,	27		
2 Sept.	9 Nov.	68	66	Fourth
1 ,,	1 ,,	61		
5 ,,	14 ,,	70		

* For the fifth generation reference should be made to Table 11.

(3-day period), and thus the daily emergence figures were entered in the records. In Text-fig. 2 these numbers during the fumigation period are entered as horizontal lines.

Numbers of midges. The average numbers of midges emerging from eight plants in the glasshouse per month are shown in Text-fig. 3 and the total numbers from these plants in Table 7.



Text-fig. 3. Average monthly emergence numbers on individual plants in an unheated glasshouse. ■ represents the initial oviposition on the eight plants.

The averages are obtained by dividing the total numbers of midges by the number of plants from which midges are emerging, i.e. in June by 2, July–August by 4, September–October by 5, November–February by 6, and from March onwards by 8.

Under the conditions of the experiment, i.e. allowing fresh plants to become infested at the commencement of each flight of midges, the population in 1938 worked up to a peak in September. By August, 4 months after the initial infestation, it was high and continued high till the end of November. In December it fell rapidly, reaching a low ebb in January. The numbers started to increase in February and continued to do so up to August when the work was brought to a close. If one considers only one of the two plants originally infected in March–April, the population peak was reached in November.

TABLE 7. *Numbers of midges emerging per month* 1938–9 *in the unheated glasshouse on eight plants*

1938		1939	
June	241	Jan.	127
July	333	Feb.	174
Aug.	1046	Mar.	340
Sept.	1887	Apr.	524
Oct.	1259	May	486
Nov.	1438	June	2232
Dec.	380	July	470
		Aug.	3367

Numbers of midges per plant (Table 8). The numbers of midges which a single plant maintained under these conditions of management (unheated glasshouse, etc.) is large. On one plant when five generations had been completed 4502 midges had developed; while on another the equivalent number was 1525. These numbers are probably higher than would occur among uncovered plants, as some of the females would be sure to fly away to neighbouring plants, but they are lower than may be possible since the females were taken out each day before completion of oviposition. They show, however, that even these numbers are not sufficient to cause the death of plants in every case. The plants even flowered, but the leaves were badly disfigured by galls, the flower stems were distorted and the flowers themselves malformed (Pl. 3, figs. 3, 4).

TABLE 8. *Rate of increase starting from five and one midges respectively under unheated glasshouse conditions*

	No. of generation					
Parent females	First, June	Second, July-Aug.	Third, Sept.-Oct.	Fourth, Nov.-Feb.	Fifth, Mar.-May	Total
5	151	513	1755	1546	537	4502
1	90	321	576	352	188	1525

Enemies. Frequently during the summer months a large red velvety mite was observed preying on midge eggs and occasionally on females as they were emerging. The mites were never seen to attack the galls, but possibly they feed on minute larvae before they are covered by plant tissue.

A more serious enemy was the earwig. These insects eat out the galls on the leaves with their contents, leaving distinct holes where the galls have been. In spite of precautions taken to exclude them from the cages earwigs kept on entering and exerted a considerable effect on the numbers of galls producing mature midges. Apparently a single earwig could account for about twenty galls from one day to the next, and they were most diligent in this work during the hours of darkness. Unfortunately, earwigs cannot be considered as a biological control of the chrysanthemum midge owing to their well-known propensity for damaging the chrysanthemums themselves.

A Pentatomid and an Anthocorid bug also were found at times sucking the eggs and the ovipositing female midges.

C. *When transferred from laboratory to glasshouse and vice versa*

It was considered useful to compare the time taken from oviposition to the first appearance of adult midges when the midges were transferred from the laboratory outside to the unheated glasshouse, at different seasons of the year, since this would give information as to the possibility of there being slow and fast developing strains. More important, however, it would show the rate of establishment of newly liberated midges, since the unheated glasshouse conditions resembled fairly closely those obtaining in the open, and this information would be useful in cases of fresh introductions into England from abroad. Table 9 gives the results when midges were taken from the laboratory to the glasshouse and Table 10

TABLE 9. *Dates of oviposition, first emergence of adult midges and the number of days from oviposition to appearance of first midge of various generations when taken from the laboratory out to the glasshouse. Asterisked entries indicate the variety of chrysanthemum used was September White, in all others Cranford Yellow*

	Oviposition	First adult midge	No. of days	Season of year	Mean
Controls (in laboratory)	26 Mar.	9 May	44	Mar.	44
,,	22 ,,	5 ,,	44		
In glasshouse	27 ,,	7 June	72		71
,,	1 Apr.	9 ,,	70		
Controls (in laboratory)	9 May	15 ,,	37	May	39
,,	5 ,,	15 ,,	41		
,,	7 ,,	15 ,,	39		
,,	9 ,,	17 ,,	39		
In glasshouse	6 ,,	20 ,,	45		48
,,	10 ,,	30 ,,	51		
Controls (in laboratory)	16 June	20 July	34	June	33
,,	15 ,,	17 ,,	32		
In glasshouse	16 ,,	23 ,,	37		38
,,	17 ,,	26 ,,	39		
Controls (in laboratory)	19 Aug.	30 Sept.	42	Aug.	43
,,	20 ,,	30 ,,	41		
,,	25 ,,	10 Oct.	46		
In glasshouse	20 ,,	8 ,,	49		49
Controls (in laboratory)	1 Oct.	19 Nov.	49	Oct.	47
,,	2 ,,	19 ,,	48		
,,	5 ,,	18 ,,	44		
In glasshouse	3 ,,	28 Feb.	148		148
Controls (in laboratory)	19* Nov.	2 ,,	75	Nov.	75
,,	19* ,,	2 ,,	75		
In glasshouse	23* ,,	21 Apr.	149		149

the results when they were taken from the glasshouse into the warmer conditions of the laboratory. Immediately the midges were taken outside they developed at a slower rate and vice versa.

Midges were taken outside (see Table 9) in late March, May and June and again in August, October and November. Starting in March, midges ovipositing out in the glasshouse took 71 days to produce the beginning of the new flight of midges compared with 44 days necessary in the laboratory. For those ovipositing in May the period was on the average 48 days compared with 39, those in June 38 compared with 33, those in August 49 compared with 43, those in October 148 compared with 47, and those in November 149 days compared with 75 (on September White variety).

Similarly, midges brought into the laboratory from outside in the glasshouse in June quickened their speed of development (see Table 10). Instead of taking 43 days on the average to the commencement of the flight only 33 days were necessary.

TABLE 10. *Dates of oviposition, first emergence of adult midges and the number of days from oviposition to appearance of first midge of generation when taken from glasshouse into laboratory*

	Oviposition	First adult midge	No. of days	Season of year	Mean
Controls (in glasshouse)	7 June	19 July	42	June	43
,,	9 ,,	20 ,,	41		
,,	8 ,,	24 ,,	46		
,,	10 ,,	22 ,,	42		
In laboratory	9 ,,	12 ,,	32		33
,,	9 ,,	11 ,,	33		

3. On other varieties

Besides studying the biology of the midge on Cranford Yellow, other varieties of chrysanthemum were also used in order to ascertain if there were distinct breeding rates on different varieties. Table 11 gives the results which can be compared with those given in Tables 2 and 6.

TABLE 11. *Dates of oviposition, first emergence of adult midges and the number of days between oviposition and the appearance of the first adult midges on different varieties of chrysanthemum. Asterisked entries indicate that the experiments were conducted in the laboratory*

Variety of chrysanthemum	Oviposition	First adult midges	No. of days
Crimson Circle	11 May	24 June	44
,,	12 ,,	17 ,,	36*
"Rose Pink"	11 ,,	22 ,,	42
September White	12 ,,	24 ,,	43
Korean Apollo	20 ,,	13 July	54
Crimson Circle	17 June	2 Aug	46*
September Pink	20 July	20 ,,	31*
,,	28 ,,	4 Sept.	38
Phoenix	23 ,,	17 Aug.	25*
,,	26 ,,	2 Sept.	38
September White	3 Oct.	17 Nov.	45*
,,	4 ,,	17 Feb.	136
,,	2 Nov.	8 Mar.	126
,,	2 ,,	20 ,,	138
Goacher's Single	7 ,,	2 Apr.	146
Dobbie's Brilliant	8 ,,	21 Mar.	133
September White	19 ,,	2 Feb.	75*
,,	19 ,,	2 ,,	75*
,,	23 ,,	21 Apr.	149

It is clear that the midge breeds at the same rate on the different varieties used whether in the laboratory or in the unheated glasshouse. There is an indication, however, that it may breed slightly slower on Korean Apollo.

Appearance of the galls. The time which elapsed between oviposition and the first appearance of the galls is given in Table 12. In the September White experiments set up on

2 November, one plant showed galls in 35 days and the other in 72 days. In the former the plant had a rapidly growing late-flowering stem, while the latter had only the fresh young growth at the base which was growing very slowly. The plants of Goacher's Single and Dobbie's Brilliant were in the same state of slow growing basal shoots.

TABLE 12. *Period in days from oviposition until galls were clearly visible on different varieties of chrysanthemum. Asterisked entries indicate that the experiments were conducted in the laboratory*

Variety of chrysanthemum	Oviposition	Appearance of galls	Period in days
Mauve Queen (two)	8 Mar.	23 Apr.	48
,,	8 ,,	26 ,,	51
"Rose Pink"	11 May	8 June	28
Crimson Circle	11 ,,	8 ,,	28
,,	12 ,,	27 May	15*
September White	12 ,,	8 June	27
Korean Apollo	20 ,,	20 ,,	31
September White	3 Oct.	25 Oct.	22*
,,	2 Nov.	7 Dec.	35
,,	2 ,,	13 Jan.	72
Goacher's Single	7 ,,	5 Feb.	90
Dobbie's Brilliant	8 ,,	17 Jan.	70
September White	19 ,,	19 Dec.	30*
,,	19 ,,	19 ,,	30*
,,	23 ,,	5 Mar.	102

4. On other species

The chrysanthemum midge was also reared in the glasshouse on *C. indicum* L., one of the forebears of all commercial autumn-flowering chrysanthemums, on *C. indicum* var. *azaleoides* which appears in trade catalogues as *C. azaleanum* and on *C. rubellum* Sealy. The last-named species is the *C. erubescens* of common usage. The results are shown in Table 13. The midge bred normally on these three plants and photographs of their galls on *C. indicum* and *C. rubellum* have been published (Barnes, 1939). The period taken from oviposition to the commencement of the flight of the midges appears to be slightly longer than on ordinary autumn-flowering chrysanthemums (see Tables 6, 11 and 13).

TABLE 13. *Dates of oviposition, first emergence of adult midges and the number of days between oviposition and the appearance of the first adult midges on different species of chrysanthemum. All entries refer to experiments in the glasshouse*

Species of chrysanthemum	Oviposition	First adult midge	No. of days
C. indicum L.	22 Mar.	5 June	71
,,	26 ,,	2 ,,	67
,,	6 May	25 ,,	50
,,	11 ,,	25 ,,	45
C. rubellum Sealy	10 ,,	1 July	52
,,	12 ,,	2 ,,	51
,,	18 ,,	5 ,,	48
,,	18 ,,	9 ,,	52
C. indicum var. *azaleoides*	20 ,,	19 ,,	60

Appearance of the galls. The periods after oviposition before the galls were clearly visible under conditions in the glasshouse are shown in Table 14.

TABLE 14. *Period in days from oviposition until galls were visible on miscellaneous species of chrysanthemum*

Species of chrysanthemum	Oviposition	Appearance of galls	Period in days
C. indicum	22 Mar.	14 May	53
,,	26 ,,	14 ,,	49
,,	6 May	20 June	45
,,	11 ,,	20 ,,	40
C. rubellum	10 ,,	20 ,,	41
,,	12 ,,	20 ,,	39
,,	18 ,,	20 ,,	33
,,	18 ,,	20 ,,	33
C. indicum var. *azaleoides*	20 ,,	30 ,,	41

5. SEX RATIO

A few experiments were made with isolated females on *C. indicum* and several varieties of autumn-flowering chrysanthemums in order to gain some idea of the sex ratio (see Table 15). Most of these experiments were conducted in the glasshouse, those on *C. indicum* being started late in March and those on the other chrysanthemums in May. The experiment with Crimson Circle was done in the laboratory and produced sixty-five offspring. Only families of forty or more individuals are included. It can be seen that, excluding *C. indicum*, the sex ratio is about 36 : 64 with two notable exceptions: in one the ratio was 20 : 80, in the other 84 : 16. Previous experience with gall midges indicates that a similar state of affairs occurs among other species. The most common sex ratio for the chrysanthemum midge in the experience of the writer is, however, about 36 : 64.

TABLE 15. *Progeny of single mated females*

Variety of chrysanthemum	Males	Females	Total offspring	Sex ratio
C. indicum	23	28	51	45 : 55
,,	25	21	46	54 : 46
Cranford Yellow	31	59	90	34 : 66
,,	10	40	50	20 : 80
Crimson Circle	22	43	65	34 : 66
"Rose Pink"	39	61	100	39 : 61
Crimson Circle	63	12	75	84 : 16
September White	14	26	40	35 : 65

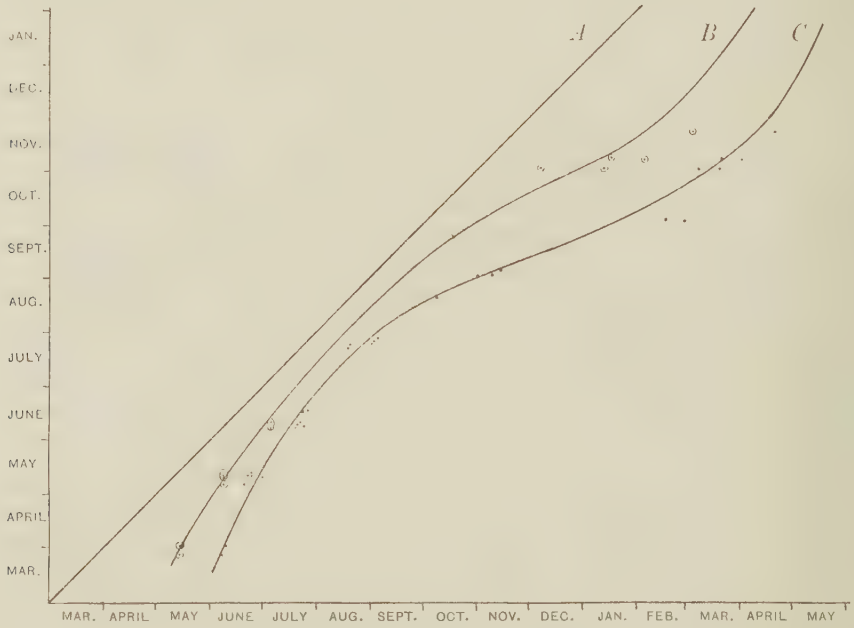
6. FIELD NOTES AND PREDICTION OF APPEARANCE

When opportunity has arisen the midge has been studied on commercially grown chrysanthemums. In mid-July 1938 all stages of the midge were plentiful out-of-doors in Sussex. In early December of the same year newly emerged adult midges and eggs were found under glass in Essex. In another area incipient galls were found late in December. In early January 1939 galls containing living larvae and parasitic larvae were found out-of-doors in the south of England. In May, both on chrysanthemum stools and cuttings large numbers of galls were present under glass in Sussex and a few eggs were found as well as empty pupal cases. In addition a few galls were found on chrysanthemums in the open.

These notes confirm the conclusion formed that the conditions under which the experi-

ments were carried out in the unheated glasshouse at Rothamsted Experimental Station were closely similar to those obtaining in commercial chrysanthemum-growing in England.

Using the data obtained under unheated glasshouse conditions concerning the time that must elapse between egg-laying and the appearance of the galls and the first adult midges, it has been possible to draw curves from which can be read the period of the year when galls and midges may be expected from eggs laid at any time of the year and vice versa. These curves are shown in Text-fig. 4, in which A represents the dates of oviposition, B those of gall appearance and C the dates on which midges may be expected. These curves are useful since from them it is possible to reckon backwards or forwards the dates on which eggs,



Text-fig. 4. Curves showing the expected dates of the appearance of galls and adult midges under unheated glasshouse conditions.

galls or adult midges may be expected. Thus, if galls are noted on a certain date, one can tell when to expect midges; or, if eggs are found, the dates on which galls should appear. The curves are based on the conditions encountered during 1938 and results obtained so far during 1939 show that the curves for 1938 approximate very closely to those for 1939.

7. Important points in the biology

Freshly emerged adult midges may be found throughout the year. This is shown by the fact that after 19 July 1938, when the stock in the glasshouse had become established, there were very few days on which no midges emerged. The emergences went on almost unbroken in spite of the comparatively few infested plants, the moderate numbers of midges reared and the low temperatures which occurred in the glasshouse in late December 1938 and

early January 1939. A few emergences actually occurred when the temperature was below freezing.

This prolonged emergence period is due to the great overlapping of the generations. Under laboratory conditions, which can be considered as sunless (the windows being north and east), cool in the summer and not extremely cold during the winter, there were eight generations in the period January 1938–February 1939. In the unheated glasshouse which was shielded from sunshine during the height of the summer and closed during the coldest part of the winter, there were five generations from the end of March 1938–March 1939.

The period from oviposition to the appearance of the first progeny varied according to the time of the year, the shortest period being in the height of the summer (Text-fig. 5). Thus, in the laboratory, the average period for the first generation was 77 days, for the second 44, for the third 39, for the fourth 34, for the fifth (*c.* 18 July–14 August) 29, for the sixth 43, for the seventh 47 and for the eighth (*c.* 20 November–14 February) 83 days. In the glasshouse the average period for the first generation in 1938 was 71 days (in 1939 this period was 66 days), for the second 43 (in 1939 this period was about 42 days), for the third (*c.* 24 July–19 August) 30, for the fourth 66 and for the fifth (*c.* 2 November–8 March) 136 days.

Emergence takes place early in the day. More midges emerge on bright than dull days and more on warm than cold days.

The adult midges usually die the day they emerge. Five days is the record of survival observed.

Oviposition starts soon after emergence and very largely is completed on the same day as emergence. Very occasionally in the summer (e.g. during cold sunless spells) egg-laying continues on the second day and in the winter is prolonged on occasion to the third.

Female midges will oviposit in glass tubes, on muslin and in the soil when no plant material is available.

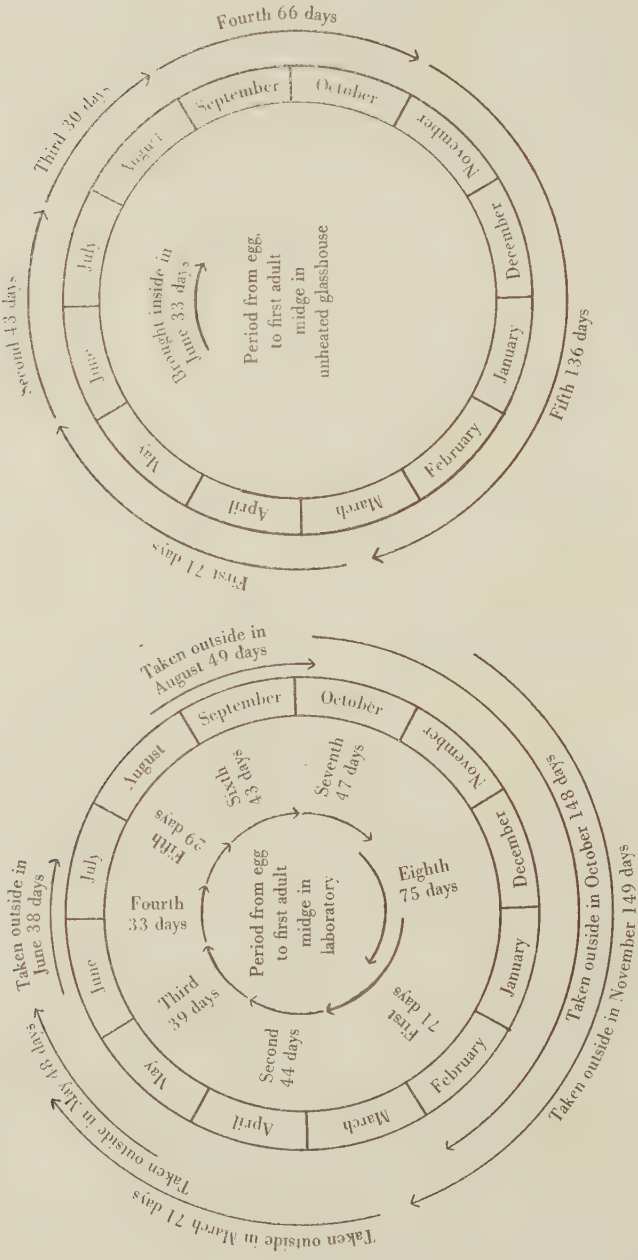
The eggs are tucked in among the buds, the folds of the young leaves and the sepals of the flower buds; occasionally they are also laid on the green stems.

The larvae hatch in 3–14 *days according to the weather.* American workers have found that the larvae become completely enclosed in the plant tissue within 24 hr. of hatching. A definite gall later develops.

The gall is closed and the larvae are thus protected.

Galls become readily visible in 25 (*June–July*) *to* 102 *days* (23 *November–5 March*) *in the unheated glasshouse. During July this period is probably reduced to* 16 *days. In the laboratory galls became visible during June in* 13 *days. In the winter this pre-gall period is sometimes greatly lengthened. Galls take longer to appear from eggs laid in the autumn on the slow growing basal shoots than from eggs laid at the same time on the rapidly growing late flowering stems.* During the winter the galls will suddenly appear and rapidly become full sized, giving the impression that the breeding rate is rapid. In some cases, however, the galls do actually appear quickly after oviposition during the winter, particularly if the eggs are laid on late flowering shoots.

The pupal period varies from 6–7 *days to several weeks.* When the galls have appeared quickly during the winter the pupal stage is lengthened. On one plant pupae were present for at least 4 weeks before emergence, possibly for 6 weeks. The shorter periods naturally occur during the summer.



Text-fig. 5. Schematic representation of the speed in days of development from egg to midge. A, in laboratory and when taken outside to unheated glasshouse; B, in unheated glasshouse and when brought inside to laboratory.

The shortest time from egg to adult observed in the unheated glasshouse was 26 *days, the longest* 149. The former was during the summer and the latter during the winter.

Emergence of adult midges was observed to take place up to the end of February from apparently quite dead old flowering shoots.

The midge can survive out-of-doors during the winter. This has been noted in England, Scandinavian countries and in the U.S.A.

The main difference between the biology of the insect in the U.S.A. (Weigel & Sanford, 1920; Hamilton, 1924) and in England is the absence of the aestivation period during the summer months in England.

8. Notes on control

Since the midge is not yet established in England all control measures are aimed at total eradication.

The adult midges are susceptible to nicotine, but since adult midges emerge practically every day during the year, fumigation would only eradicate this pest if it were done every night which is impracticable. Spraying with nicotine, although it kills adult midges and eggs, would similarly have to be done about every 3 days during the warm weather as the larvae hatch from the eggs in about 3 days and within 24 hr. are out of reach of the spray, having entered the plant tissue. Moreover, one could never be certain of obtaining a 100% kill of eggs.

Dipping cuttings in nicotine will help in checking midge increase. Table 16 gives the results of experiments carried out in the spring of 1939. Midges were allowed to oviposit on chrysanthemum shoots in early March and early April. At intervals after the eggs had been laid the twenty shoots were dipped in nicotine (95–98%) 1 : 800 with 1 oz to 1 gal. soft soap, each cutting being immersed in the solution for 5 sec. Six shoots were kept as controls. The cuttings were then rooted and later examined for galls.

Table 16. *Effect of dipping in nicotine-soft soap on eggs and young larvae*

	Av. no. of galls		
	Per dipped cutting	Per control	Variety
1. Eggs (0–1 day old)	0·25	—	Thanksgiving Pink
2. Eggs (0–2 days old	1·8	18·5	Mauve Queen
3. Eggs (8–10 days old)	0	—	Mauve Queen
4. Eggs (9–11 days old)	0·75	3	Mrs Arkwright
5. Larvae (up to 4 days old)	7·5	*c.* 64	Mauve Queen

No difficulty was found in rooting the dipped cuttings, but there seemed to be a slight retarding effect. Although the numbers of midges were greatly reduced by dipping the eggs, it was not to be expected that every egg would be destroyed. When young larvae were present, a higher proportion survived.

On the other hand, total eradication should be possible if, after flowering, the plants were cut down to ground level, transferred to a closed uninfected glasshouse and fresh growth allowed to develop, from which cuttings would eventually be taken. Care would be necessary to avoid bringing old leaves and plant refuse into the uninfected house as it has been shown that midges will emerge from dead leaves.

Experiments were carried out to test this method of control. Midges were allowed to oviposit on chrysanthemum plants which were later cut down to various levels. In one case (Exp. 4) midges were given the opportunity to oviposit on the plant after it was cut down. The results of these experiments are shown in Table 17.

TABLE 17. *Effect of cutting down the chrysanthemum plants*

Extent of cutting	Degree of infection	No. of galls or adults reared on the new growth	Variety
1. Complete (except for old flowering stalk)	13 ♀♀ ovipositing before cutting	0	Mauve Queen
2. Complete (except for old flowering stalk)	9 ♀♀ ,, ,,	0	Thanksgiving Pink
3. Complete	9 ♀♀ ,, ,,	0	Mauve Queen
4. Rough cut (¾ in. left)	11 ♀♀ ovipositing after cutting	0	September White
5. Rough cut (½ in. left)	13 ♀♀ ovipositing before cutting	0	Mrs Arkwright
6. Uncut (except for terminal few inches)	13 ♀♀ ,, ,,	1	Mauve Queen

Total eradication was obtained with the exception of one case (Exp. 6) in which the uncut plant had only the terminal few inches of each shoot removed. Even when midges were given the opportunity of ovipositing on the remaining ¾ in. of stems, none developed; under these conditions, the midges were sooner or later caught on the exuding sap which apparently was most attractive. New growth developed on all the varieties used, but in some cases not so freely as on others.

9. SUMMARY

The life history of the chrysanthemum midge has been worked out at Harpenden, England, both under laboratory and unheated glasshouse conditions. Important points have been emphasized in § 7.

The midge breeds continuously throughout the year, the generations following each other more rapidly as the hottest weather approaches. Under unheated glasshouse conditions five generations occurred between March 1938 and March 1939. Considerable overlapping takes place. There was no evidence of hibernation or aestivation. Emergence has been observed when there was frost on the plants, but the threshold temperature for mating and egg-laying appears to be slightly above freezing-point. No ceiling temperature or upper limit for breeding was encountered.

Field notes have shown that the midge behaves in commercial nurseries in much the same way as in the experiments conducted in the unheated glasshouse throughout the year.

For total eradication it is shown that fumigation and spraying are not economical. Dipping the cuttings in nicotine-soft soap solution greatly reduces the numbers of developing midges. The most hopeful method of eradicating the pest seems to be cutting down the entire plant, then isolating the cut down stools and, subsequently, using the new growth for cuttings.

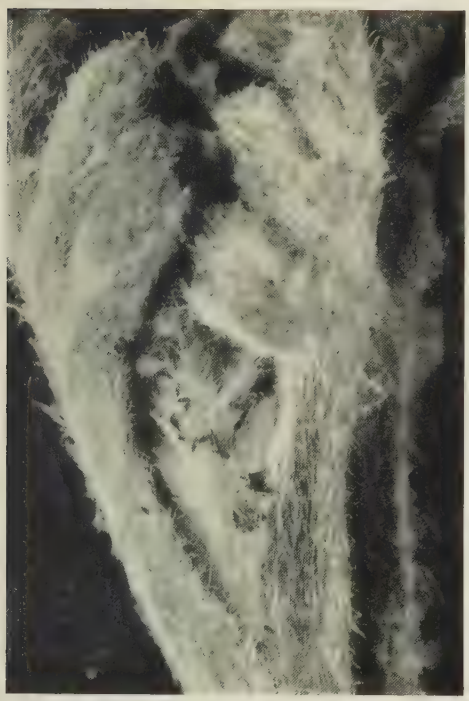


Fig. 1.

Fig. 2.

Fig. 3.

Fig. 4.

REFERENCES

Barnes, H. F. (1939). Some gall midge species and their host plant range. *Ann. app. Biol.* **26**, 318–47.

Hamilton, C. C. (1924). The biology and control of the chrysanthemum midge (*Diarthronomyia hypogaea* F.Lw.). *Bull. Md agric. Exp. Sta.* no. 269.

Weigel, C. A. & Sanford, H. L. (1920). Chrysanthemum midge. *Bull. U.S. Dep. Agric.* no. 833.

EXPLANATION OF PLATE 3

Fig. 1. Eggs of chrysanthemum midge in terminal leaf bud. (Reproduced from *Adv. Leafl. Minist. Agric. Fish. Lond.*, 286 (edition 1939), by permission of the Controller of H.M. Stationery Office.)

Fig. 2. Galls on leaf. (Photo. A. S. Buckhurst.)

Fig. 3. Galls on flowering stem.

Fig. 4. Reduction in growth caused by midge attack.

(*Received* 31 *May* 1939)

Addendum (30 October 1939)

Evidence has been accumulating (Barnes, 1939) that the chrysanthemum midge of commercial chrysanthemums is different from the form (*Diarthronomyia hypogaea*) described by F. Loew from ox-eye daisy. It had been hoped however to carry out reciprocal studies on *Di. hypogaea* obtained from ox-eye daisy before making a final decision. Recently, however, O. Ahlberg (*Ent. Tidskr.* **60**, 1939, 274–8) has independently proposed the new name *Di. chrysanthemi* for the midge attacking commercial chrysanthemums and so, in future the chrysanthemum midge should be known as *Diarthronomyia chrysanthemi* Ahlberg (=*hypogaea* F.Lw. of Felt, *inter aliis*, nec *hypogaea* F.Lw. of Loew).

THE DETECTION OF WOOD-BORING INSECTS BY MEANS OF X-RAYS

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(With Plates 4 and 5 and 1 Text-figure)

INTRODUCTION

IN the course of investigations at the Forest Products Research Laboratory upon the biology and control of wood-boring insects, the need has become apparent for a reliable means of detecting insects in timber or furniture, in the absence of outward and visible signs of their presence, without cutting up the material concerned. In this connexion, a characteristic feature of infestation by the death-watch beetle, *Xestobium rufovillosum* De G., and the common furniture beetle, *Anobium punctatum* De G., is the cessation of attack before all the available timber has been destroyed. In such circumstances the application of control measures may be unnecessary and could be avoided by showing that living insects were no longer present.

During the past few years this problem of detecting insects hidden within timber has been the subject of experiment at Princes Risborough in collaboration with other Laboratories. Two methods have been used. One, examined in co-operation with the National Physical Laboratory, explored the possibilities of applying microphone and amplifier technique to detect the presence of wood-boring larvae by listening to the faint sounds made by them whilst feeding or moving about within the timber (Colebrook, 1937). The other method concerned the use of X-ray photography, and is the subject of the present paper, which presents the results of a joint investigation by the Forest Products Research Laboratory and Messrs Ilford, Ltd. The effect of X-rays on the insects has not been examined in the course of this work.

LITERATURE

Published accounts of the use of X-rays for detecting insects in materials are few, and little information is available from past work on the practical value of the treatment. Yuasa (1926), in Japan, advocated the method for revealing such insects as borers in plant material subject to quarantine, whilst Fenton & Waite (1932) in the U.S.A. demonstrated by X-ray photographs that it was possible to detect living and dead larvae of the pink bollworm, *Platyedra gossypiella* Saund., in cotton seeds. Further work on these lines was carried out in Russia by Shevchenko (1937), who used soft X-rays to examine the seeds of thirty-three species of cultivated crops and forest trees. The presence of insects was readily determined and the viability of the seeds was not affected.

The first definite attempt, of which a published account is available, to detect insect

attack and fungal decay in living trees and felled logs was made by Maloy & Wilsey (1930). Their work showed that although the finer details of the structure of wood were not revealed, the extent of decay and its distribution were shown. Radiographs of 1 in. slabs cut from logs, the species of which is not mentioned, demonstrated the presence of two unidentified wood-boring larvae, which appear from the figure in their paper to be of the family Buprestidae. These workers suggest that X-ray examination of thin slabs and sections of trees would be of value for laboratory studies of disease and decay. Worschitz (1933), in discussing the use of X-rays for examining the structure and physical properties of wood, draws attention to their value for detecting various types of defects, and remarks that the tunnels of wood-boring insects can easily be located, particularly if the X-rays pass through the wood more or less parallel to the direction of the tunnels. According to Seeman (1937), however, past work on the radiography of wood, as for instance in the inspection of wooden aeroplanes during the War of 1914–18, laid emphasis more upon the parts considered as assemblies than upon the quality of the original solid wood. He mentions an unpublished record from England of an X-ray examination of oak attacked by the death-watch beetle, but we have been unable to trace the origin of this work.

Arising out of some preliminary experiments undertaken in 1930–1 by Mr W. W. Barkas, Officer in Charge of the Section of Timber Physics at the Forest Products Research Laboratory, in co-operation with the X-ray Department, University College, London, and subsequently with the National Physical Laboratory, arrangements were made in 1935 for more exhaustive tests on the use of X-rays for detecting wood-boring insects in timber, to be undertaken by the Scientific Department of the Courtauld Institute of Art, University of London (Rees-Jones & Ritchie, 1937). It was shown that larvae of *Lyctus* powder-post beetles can easily be detected in samples of oak and ash $\frac{1}{2}$–$\frac{5}{8}$ in. thick, using very soft X-radiation (16 kVp., 10 mA.). Successive radiographs showed changes in position of living larvae, and the authors suggested and checked spectrographically that the relatively great absorption of the rays by the larvae is due to concentration within their tissues of the mineral salts present in the wood. The degree of mineralization in the tissues of different wood species, as revealed by X-rays, has been discussed by Pasinetti (1938), who, describing the use of X-rays for revealing structural alterations and defects in wood due to mechanical injury of the tree and to fungal attack, comments favourably upon the value of the method for detecting the presence of insect tunnels within timber.

Present investigation

The results of these past investigations on wood, although encouraging, showed the need for further experiment, for which facilities became available in 1937.

A joint programme of work was prepared, and the Forest Products Research Laboratory undertook to provide insect-infested timber and carry out the necessary detailed examination of the samples after X-ray treatment, whilst the development of a suitable radiographic technique was left to Messrs Ilford, Ltd.

Material

The samples used ranged in thickness from $\frac{3}{16}$ in. plywood to 5 in. thick boards, and also included sections of varying dimensions cut from oak structural timbers from buildings. The samples were attacked by one or other of the following wood-boring insects: the death-

watch beetle, *Xestobium rufovillosum* De G.; the common furniture beetle, *Anobium punctatum* De G.; *Lyctus* powder-post beetles; and a longhorn beetle, *Isotomus speciosus* Schneider. The extent of damage varied in different samples according to the insect and the type of injury. Further details of the methods used, the wood species concerned, the dimensions of the individual samples and their condition are given below, where the results of the work are summarized.

Technique

As wood consists almost entirely of carbon, oxygen and hydrogen, its absorption coefficient to X-rays is extremely small, and in order to obtain adequate contrast for the detection of insect tunnels, X-rays of very low kilovoltage must be used. Wood-boring larvae can be detected more easily, since, as shown by Rees-Jones & Ritchie (1937), inorganic materials within the wood are concentrated in the larval tissues which are consequently of higher absorption to X-rays than the wood itself.

The X-ray apparatus available consisted of a Philips' Metalix 6 kW. X-ray tube operated on a half-wave rectified circuit. The penetrating power of X-rays increases as their wavelength decreases and hence as the kilovoltage at which they are generated increases; the kilovoltage at which the tube is operated is therefore used as a qualitative guide to the penetrating power of the X-rays.

The sphere-gap calibration previously made on the X-ray apparatus had not been carried in detail below 30 kVp. In order to estimate lower kilovoltages the calibration curves had to be extrapolated. This renders kilovoltages lower than 30 kVp. subject to appreciable error, but the extrapolated portions of the curves agreed well with a number of isolated sphere-gap readings in the range 20–30 kVp., and it is considered unlikely that even in the lowest kilovoltages quoted the error can exceed ± 4–5 kVp.

The specimen under investigation was placed on top of a naked Ilfex film resting on a lead-covered table, and scattered radiation from surrounding objects was completely eliminated. The X-ray room was illuminated through safe-lights; light from the filament of the X-ray tube was prevented from reaching the film by a sheet of black paper surrounding the window of the tube, the thin aluminium filter below the tube window being removed on account of its X-ray absorption. After exposure the films were developed in Ilford Blue Label Developer.

Some difficulty was experienced in the printing of the resulting negatives. Radiographic negatives, particularly of "industrial" subjects, are notorious for their great density range, and for the impossibility of obtaining single overall prints of sufficient contrast to show local detail throughout the whole density range. The negatives obtained from the timber specimens were no exception and in some cases several prints were necessary in order to bring out the detail at different density levels in the negative. Some detail was, therefore, inevitably lost in the printing, and it must be emphasized that in analysing the results, comparisons between the numbers of insects found on cutting up the samples and those discovered by X-rays were made from the negatives and not from prints.

In every case, the best radiograph resulted from the use of the lowest kilovoltage consistent with the production of a satisfactory density within a reasonable time. The kilovoltages employed varied between 14 and 40 kVp., but in view of the high X-ray absorption by the window of the X-ray tube at these low kilovoltages, the exposures, in milliampere-seconds,

were heavy, and it is certain that by using a tube provided with a thinner window (e.g. a Grenz-ray tube), not only could these exposures be reduced but the quality of the radiographs could be further improved by the use of even lower kilovoltages. In some cases it was necessary to give more than one exposure, even with the specimen in the same orientation with regard to the X-ray tube and film —one exposure for the severely disintegrated parts and a heavier exposure to penetrate the sound wood. This generally proved far more satisfactory than increasing the kilovoltage as this usually produced too great a diminution in contrast by the time the kilovoltage was sufficiently high for the production of an otherwise satisfactory overall radiograph. The use of a Potter-Bucky diaphragm would doubtless improve the appearance of radiographs taken through thick sections provided the increase in exposure involved would not render it necessary to increase the kilovoltage appreciably. On account of the X-ray absorption in the front screen, a pair of intensifying screens could not be employed at the lower kilovoltages and with a single back screen the loss of definition was such that the improved appearance of the radiograph obtained without a screen justified the longer exposures necessary.

After being radiographed, the samples were returned to Princes Risborough, where they were cut up for detailed examination. The position and stage of development of all insects found, alive and dead, were noted and compared with those revealed on the radiographs. The results compiled from the radiographs and from counts of insects found in the samples, together with a brief description of each and the X-ray treatment given, are detailed in the following summary.

Results

Sample 1.

Hornbeam plank, $9\frac{1}{2} \times 5 \times 2\frac{1}{4}$ in., attacked by a longhorn beetle, *Isotomus speciosus* (Cerambycidae). Sound except for a few tunnels filled with tightly packed frass.

X-ray exposure. For $2\frac{1}{4}$ in. thickness: 24 kVp., 30 mA., 5 min., 30 in. For 5 in. thickness: 39 kVp., 30 mA., 80 sec., 30 in.

Insects visible in radiographs (Pl. 4, fig. 2). One beetle, easily detected through $2\frac{1}{4}$ in., but becoming less distinct through 5 in.

Insects found. One beetle, dead.

Sample 2.

Ash board, 1 in. thick, extensively attacked by powder-post beetles (Lyctidae and Bostrychidae), but not in a powdery condition.

X-ray exposure. 20 kVp., 30 mA., 4 min., 36 in.

Insects visible in radiographs. Lyctus larvae very numerous; within one small section of sample, seventeen counted and one bostrychid larva.

Insects found (in same section). *Lyctus* larvae, nineteen alive, seven dead. Bostrychid larvae, one alive. *Lyctus* beetles, one alive.

Dead larvae invisible in radiograph.

Sample 3.

Sycamore board, $\frac{1}{2}$ in. thick, extensively tunnelled by *Lyctus* powder-post beetles.

X-ray exposure. 17 kVp., 40 mA., 15 min., 15 in.

Insects visible in radiographs (Pl. 4, fig. 3). Numerous beetles and larvae which appeared to be attacked by the predacious mite, *Pediculoides ventricosus*. Radiographs very clear, probably due to thinness of sample.

Insects found. Counts of the numerous insects not made, but presence of *P. ventricosus* confirmed.

Sample 4.

Three-ply alder, $\frac{3}{16}$ in. thick, attacked by the common furniture beetle, *Anobium punctatum*. Centre ply extensively tunnelled, outer faces intact except for exit-holes. Covered on one side with white (painted) paper.

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Insects visible in radiographs (Pl. 4, fig. 2). One beetle, easily detected through $2\frac{1}{4}$ in., but becoming less distinct through 5 in.

Insects found. One beetle, dead.

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Ash board, 1 in. thick, extensively attacked by powder-post beetles (Lyctidae and Bostrychidae), but not in a powdery condition.

X-ray exposure. 20 kVp., 30 mA., 4 min., 36 in.

Insects visible in radiographs. Lyctus larvae very numerous; within one small section of sample, seventeen counted and one bostrychid larva.

Insects found (in same section). *Lyctus* larvae, nineteen alive, seven dead. Bostrychid larvae, one alive. *Lyctus* beetles, one alive.

Dead larvae invisible in radiograph.

Sample 3.

Sycamore board, $\frac{1}{2}$ in. thick, extensively tunnelled by *Lyctus* powder-post beetles.

X-ray exposure. 17 kVp., 40 mA., 15 min., 15 in.

Insects visible in radiographs (Pl. 4, fig. 3). Numerous beetles and larvae which appeared to be attacked by the predacious mite, *Pediculoides ventricosus*. Radiographs very clear, probably due to thinness of sample.

Insects found. Counts of the numerous insects not made, but presence of *P. ventricosus* confirmed.

Sample 4.

Three-ply alder, $\frac{3}{16}$ in. thick, attacked by the common furniture beetle, *Anobium punctatum*. Centre ply extensively tunnelled, outer faces intact except for exit-holes. Covered on one side with white (painted) paper.

X-ray exposure. 18 kVp., 30 mA., 60 sec., 36 in.

Insects visible in radiographs (Pl. 5, fig. 1). Larvae: three *Anobium*, distinct, and several very doubtful. Pupae: three of another species, probably of the family Cleridae. Beetle: one (? Clerid).

Insects found. Larvae: *Anobium*, eight alive, four dead. Pupae: two Clerid. Beetles: *Anobium.*, five dead; *Paratellus carus* (Cleridae), two alive.

One of the Clerid pupae visible in the radiograph had changed to an adult before the sample was cut up. Dead *Anobium* beetles were not detected by the X-rays, nor were several *Anobium* larvae, dead or alive. In other radiographs of a similar sample of plywood, four Clerid larvae, found on cutting up, could not be definitely detected. After they had been located in the samples, blurred images could then be seen in the radiographs, which in two cases at least might have corresponded with these insects. Why such larvae should have been clearly detected in one sample but be almost invisible in the other, although in a similar condition, is not clear.

Sample 5.

Oak sapwood decayed in the Laboratory by *Phellinus cryptarum* prior to infestation by the death-watch beetle, *Xestobium rufovillosum*; 1¾ in. thick, severely attacked and in a powdery condition.

X-ray exposure. 20 kVp., 30 mA., 3 min., 30 in.

Insects visible in radiographs. Larvae: seven. Beetles: two or possibly three, one being indistinct.

Insects found. Larvae: eight alive, two dead. Beetles: three alive, one dead.

The insects not visible in the radiographs were present in the more extensively tunnelled and powdered parts of the wood.

Sample 6.

Similar to sample 5 but not extensively tunnelled and powdered.

X-ray exposure. 18 kVp., 30 mA., 8 min., 24 in.

Insects visible in radiographs. Larvae: seven, possibly nine, two being indistinct.

Insects found. Larvae: two alive, four dead. Pupa: one alive. Beetles: two alive.

Beetles not visible in radiographs.

Sample 7.

Oak sapwood decayed in the Laboratory by *Phellinus cryptarum* prior to introduction of six half-grown death-watch beetle larvae; 1½–1¾ in. thick, not attacked before larvae introduced.

X-ray exposures. With one exception, 20 kVp., 30 mA., 4 min., 36 in. Radiographed at intervals over a period of three months.

Insects visible in radiographs (Pl. 5, fig. 2*a–e*). All larvae distinct; rate of boring determined for two larvae by comparing successive radiographs.

Insects found. Three months after introduction of larvae: four larvae alive, two dead.

The clear definition of the larvae in the radiographs was probably due to the few tunnels present, with the result that the sample was not disintegrated nor in a powdered condition.

The following samples were cut from oak structural timbers from buildings, and all showed severe damage by the death-watch beetle:

Sample 8.

Oak heartwood; base area 5–7 × 8½ in.; maximum height 7 in.; extensively honeycombed at top, but unattacked sound timber in the basal half. Before radiographing, six larvae and two living death-watch beetles were inserted in tunnels drilled in the sample to a depth of 1½ in.

X-ray exposures. Numerous radiographs were taken using a variety of kilovoltages and techniques.

Insects visible in radiographs. None, not even the inserted insects.

Insects found. In addition to those inserted, several dead beetles and three living larvae were found in the less honeycombed portions of a section of the sample which was examined in detail.

Sample 9.

Oak heartwood, 3 in. thick, extensively tunnelled, especially at top; sound wood at base. Two larvae inserted at depth of 1½ in., one in sound wood and one in honeycombed part of sample.

X-ray exposure. Through 3 in. thickness—29 kVp., 30 mA., 25 sec., 36 in.

Insects visible in radiograph. Larvae: eight, including the one inserted in the sound wood.

Insects found. Larvae: nineteen alive. Beetles: four alive, one dead.

Only a few larvae were detected by the X-rays, which failed to pick out any beetles, alive or dead.

Sample 10.

Oak heartwood, 2 in. thick. General condition similar to samples 8 and 9. Two larvae inserted at depth of 1 in., (*L* 1) from base and (*L* 2) from one face.

X-ray exposure. 29 kVp., 30 mA., 15 sec., 36 in.

Insects visible in radiographs (Pl. 4, fig. 1). One inserted larva (*L* 1) and one other were clearly visible. The second inserted larva was indistinctly visible in the negative.

Insects found. Larvae: inserted specimens and three others, alive. Beetles: three dead.

None of the dead beetles, which were found in the honeycombed portions of the sample, was visible in the radiographs.

Sample 11.

Oak heartwood, $1\frac{1}{4}$–$1\frac{3}{4}$ in. thick. Condition similar to preceding sample. Two larvae inserted at depth of $1\frac{1}{2}$ in.

X-ray exposures. 29 kVp., 30 mA., 1·5 sec., 36 in.: 29 kVp., 30 mA., 7·5 sec., 36 in.

Insects visible in radiographs. Introduced larvae only.

Insects found. Larvae: inserted specimens and one other small living larva. Beetles: one alive on surface, and one dead near surface.

Neither of the beetles nor the small larva were detected by the X-rays.

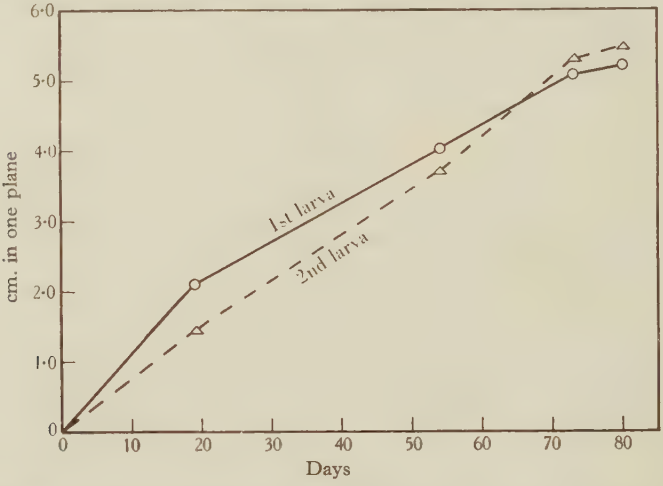
Discussion

It is generally recognized that for a cavity in the body of a material to be detectable by its shadow on a radiograph it must have a thickness not less than 1–2 % of the total thickness of the material; in unfavourable cases, e.g., in thick specimens, the cavity must form a greater percentage of the total thickness of the material. In insect-attacked timber, therefore, the minimum diameter of an empty tunnel which would be detected in a given thickness of sound wood is governed by this limitation. Tunnels packed with frass would be expected to be more difficult to detect, on account of the small difference in X-ray absorption between the sound wood and the frass. Furthermore, the more tightly packed the frass in the tunnel the more difficult it will be to differentiate it from surrounding sound wood. This is clearly shown by the radiographs (Pl. 4, fig. 2) of the plank of hornbeam containing larval tunnels of a longhorn beetle, *Isotomus speciosus*, of which a characteristic feature is the compact and tightly compressed bore dust with which they are filled. In these radiographs, however, the course of the larval tunnels can be traced by the pattern produced by what appears to be an arrangement of the bore dust in waves and whorls, indistinguishable by the naked eye. Such a mode of packing of the frass, revealed by X-rays, recalls a somewhat similar but easily visible condition in the bore dust produced by the larvae of many Buprestid species, and which, according to Escherich (1923), enables the larval tunnels of these wood-borers readily to be distinguished from those of the longhorns. Radiographic examination of the frass of the above longhorn beetle suggests, however, that such an arrangement of frass particles is not confined to the workings of Buprestid larvae, although it may be more easily visible in the tunnels of these insects.

The detection of an insect in an empty tunnel (Pl. 4, fig. 2) should be comparatively easy when the specimen is not too thick, depending chiefly upon the absorption of the rays by the insect, but when it is in a tunnel packed with frass its detection depends upon the differential absorption of insect and frass, and will become more difficult. In actual practice, however, as the above results show, it was found that the principal factor governing the detection of insects was the state of the wood itself. If this was severely disintegrated or badly tunnelled, the radiograph was confused and indistinct and it was very difficult to

locate insects, even in thin samples, while it was much easier to detect similar insects in considerably thicker sections of sound wood. For instance, samples 5 and 7 were of similar thickness, but the former was much more severely powdered than the latter; on cutting up 5, larvae not detected by the X-rays were revealed, whilst in 7, which contained only a few tunnels and was not disintegrated, all the insects present were clearly visible in the radiograph. Again, the failure of X-rays to pick out insects in wood cut from death-watch beetle-infested timbers from buildings was primarily due to its honeycombed and disintegrated condition.

Further, the results show that, in general, larvae are more readily detected than beetles. Death-watch beetles were hardly ever detected, dead or alive, nor were dead specimens of the common furniture beetle visible in radiographs of three-ply alder only $\frac{3}{16}$ in. thick. In



Text-fig. 1. Rate of boring of half-grown larvae of the death-watch beetle in decayed oak sapwood at 22° C. and 18–20 % moisture content.

$\frac{1}{2}$ in. thick sycamore, on the other hand, *Lyctus* powder-post beetles, alive and dead, were clearly picked out by the X-rays. Such variation in results must necessarily limit the accuracy with which the method can be relied upon to reveal the presence of insects, alive or dead, in all types and sizes of sample.

When insects are detected, however, use can be made of radiographs taken at successive intervals of time, to note by changes in position whether they are alive or dead, and the method might be extended to follow the development of an insect throughout its different stages. In this connexion, the condition of sample 7 made it possible to observe from a series of radiographs taken over a period of 3 months the rate of tunnelling of two death-watch beetle larvae in decayed oak sapwood at 22° C. and 18–20 % moisture content. Two of the six larvae in this sample were active during this period, and from the radiographs (Pl. 5, fig. 2*a–e*) their respective total linear movements were plotted as a function of the number of days during which the experiment was in progress (Text-fig. 1). The X-ray exposures were, with one exception, 20 kVp., 30 mA., 4 min., 36 in. In view of the relatively large focus-film distance, no correction was made for the slight geometrical magnification of the

image. It will be seen from Text-fig. 1 that both larvae maintained a fairly constant rate of progress of approximately 1 cm. per fortnight. Biological studies (Fisher, 1937) of the death-watch beetle have shown, however, that the rate of boring of the larvae and the duration of life cycle of the insect are closely related to the condition of the timber with reference to the presence and extent of fungal decay, and there are indications (Forest Products Research Board, 1938) that the resulting differences in degree of mechanical resistance offered to boring, by wood which has been decayed to a varying extent, have a direct effect upon the rate of progress of the larvae. Although the figure of 1 cm. per fortnight can, therefore, be related only to the specific conditions present in the moderately decayed sample concerned, it provides interesting evidence in support of conclusions previously reached from the biological investigations to the effect that under the conditions normally prevailing in structural timbers in a building, the progress of attack and rate of disintegration of timber infested by the death-watch beetle is extremely slow.

Conclusions and summary

The following conclusions can be drawn from the results of this investigation:

(1) The general condition of a sample of wood can readily be determined by X-ray examination. For example, the presence of insect tunnels and the extent of disintegration within a sample can be detected with ease, even when not apparent from its external appearance.

(2) In favourable cases the presence or absence of insects can be ascertained, but for the results of the examination to be conclusive the sample must not be too thick, and, above all, must not be severely disintegrated and powdered. It is not possible to state definitely the limits of thickness through which various insects can be detected; this depends primarily upon the state of the wood and, secondly, on the stage and size of the insects and the amount of frass in the tunnels in their immediate neighbourhood.

(3) When insects can be detected their movements can be followed by taking radiographs at successive intervals of time. The method might prove of value in laboratory studies of wood-boring insects when the progress of development cannot usually be determined without destructive examination of the infested samples.

(4) It appears that the practical application of X-rays for the detection of wood-boring insects will be confined to timber of small dimensions, e.g. small articles of furniture, picture panels and frames, plywood, etc., and, where cost allows, could be used for determining the efficacy of methods of control by insecticide or fumigation treatments applied to such material. The examination of structural timbers *in situ* in buildings for the detection of the death-watch beetle is in general impracticable. Such timbers are usually of large dimensions, and whilst it is reasonably certain that larvae favourably situated would be detected, a negative result would be inconclusive, and beetles would generally not be located.

(5) The X-ray equipment best suited to the radiography of wood is one with a high power output operating in the voltage range 10–40 kVp. A Grenz-ray tube or an X-ray tube having a very thin window is essential if kilovoltages below 20–25 are to be used.

The authors are indebted to Mr W. A. Robertson, Director of Forest Products Research, and to the Directors of Messrs Ilford, Ltd., for permission to publish this account of their joint investigation.

REFERENCES

Colebrook, F. M. (1937). The aural detection of the larvae of insects in timber. *J. sci. Instrum.* **14**, 119.

Escherich, K. (1923). *Die Forstinsekten Mitteleuropas*, **2**, 131.

Fenton, F. A. & Waite, W. W. (1932). Detecting pink boll-worms in cotton seeds by the X-ray. *J. agric. Res.* **14**, 347.

Fisher, R. C. (1937). Recent work on wood-destroying insects. *J. R. Soc. Arts*, **85**, 407.

Forest Products Research Board (1938). *Annual Report*, H.M.S.O., London.

Maloy, T. P. & Wilsey, R. B. (1930). X-raying trees. *Amer. Forests For. Life*, **36**, 79.

Pasinetti, L. (1938). La rontgendiagnostica applicata alla fitographia ed alla fitopatologia. *Riv. Patol. veg.* **28**, 155.

Rees-Jones, S. & Ritchie, P. D. (1937). Radiographic detection of *Lyctus* larvae *in situ*. *Tech. Stud.* **5**, 179.

Seeman, H. E. (1937). Miscellaneous applications of radiography and fluoroscopy. *Symposium on radiography and X-ray diffraction methods*. Amer. Soc. Test. Mat.

Shevchenko, M. I. (1937). Revealing seed pests by means of X-rays. [*In Russian.*] *Plant Prot.* **14**, 14–25 (with a summary in English).

Worschitz, F. (1933). L'utilisation des rayons en vue de l'étude de la qualité des bois. *Congr. int. Union For. Res. Organizations, Nancy*, 1932, pp. 459–86.

Yuasa, H. (1926). On the advantage of the X-ray examination of certain classes of materials and insects subject to the plant quarantine regulations. *Proc. 3rd Pan-Pacific Sci. Congr., Tokyo*, **1**, 1141.

EXPLANATION OF PLATES 4 AND 5

Plate 4

Fig. 1. Radiograph of part of oak beam showing two larvae of the death-watch beetle in the sounder parts of the sample. × 1. (Sample 10.)

Fig. 2. Radiograph of portion of 2¼ in. plank of hornbeam attacked by the longhorn beetle, *Isotomus speciosus*, showing larval tunnels and immature beetle in pupal chamber. × 1. (Sample 1.)

Fig. 3. Radiograph of portion of *Lyctus*-infested ½ in. board of sycamore showing beetles and larvae, some of which are attacked by the predacious mite, *Pediculoides ventricosus*. × 1. (Sample 3.)

Plate 5

Fig. 1. Radiograph of $\frac{3}{16}$ in. three-ply alder attacked by the common furniture beetle, *Anobium punctatum*. One beetle and two pupae of another species (? Cleridae) are visible. × 1. (Sample 4.)

Fig. 2 *a–e*. Radiographs showing rate of progress of two half-grown larvae of the death-watch beetle, *Xestobium rufovillosum*, in decayed oak sapwood at 22° C. and 18–20 % moisture content. × ½. (Sample 7.)

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Fig. 1.

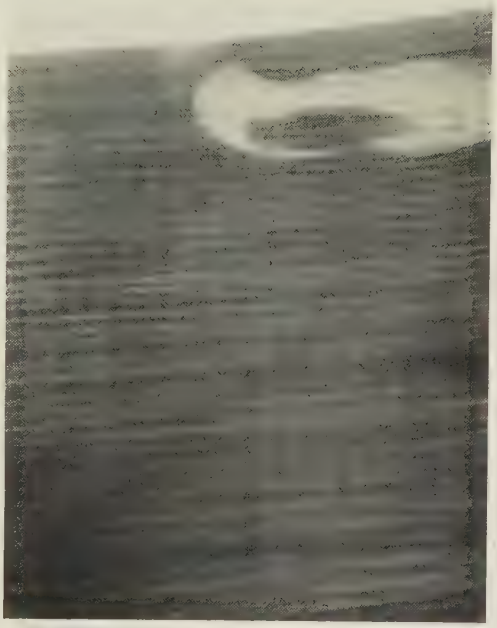


Fig. 2.

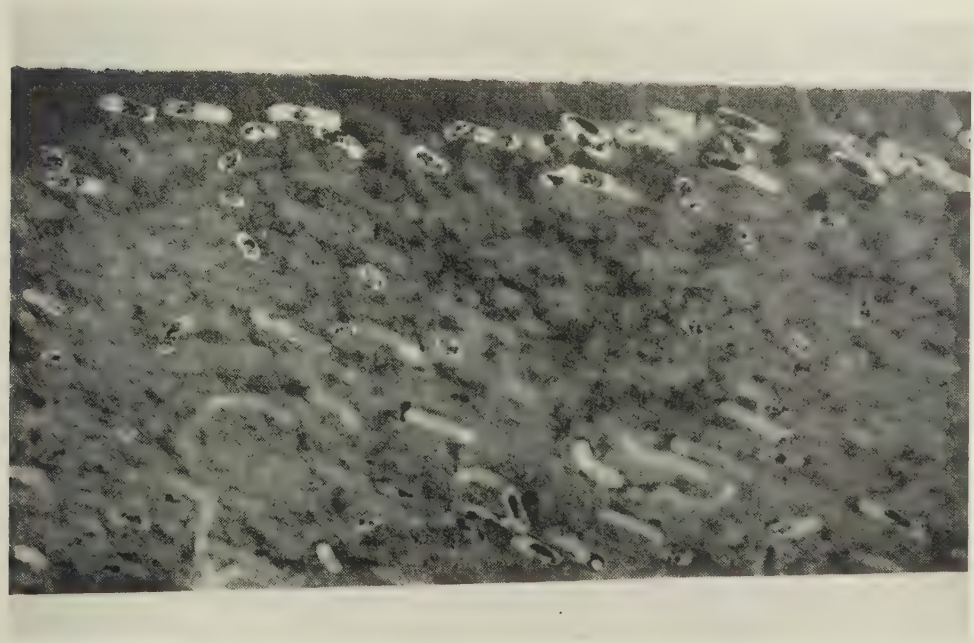


Fig. 3.

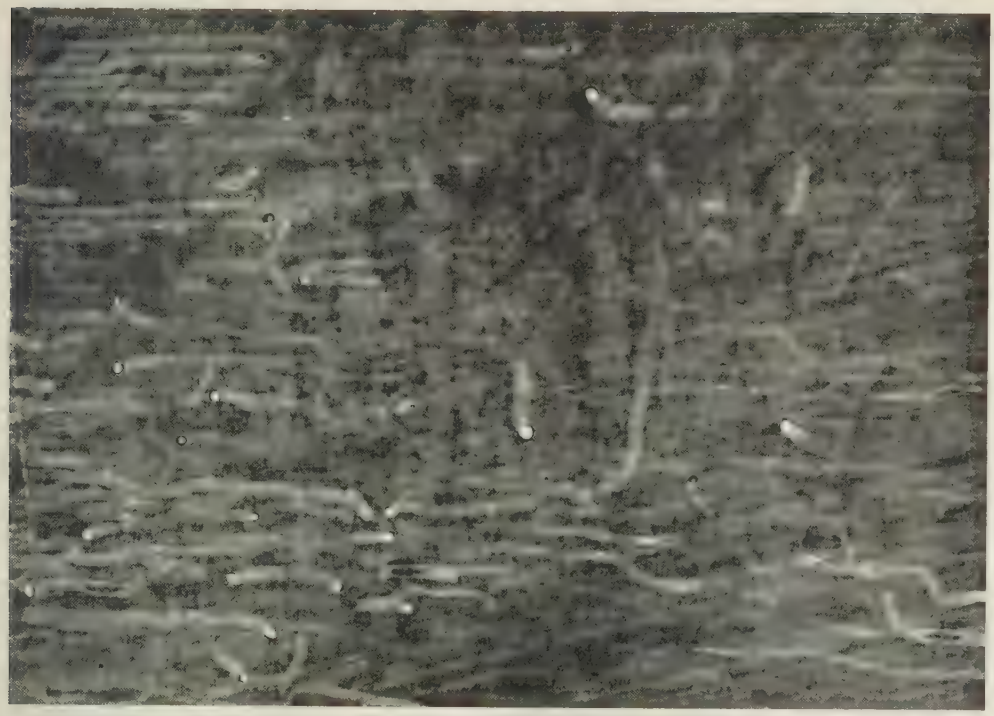
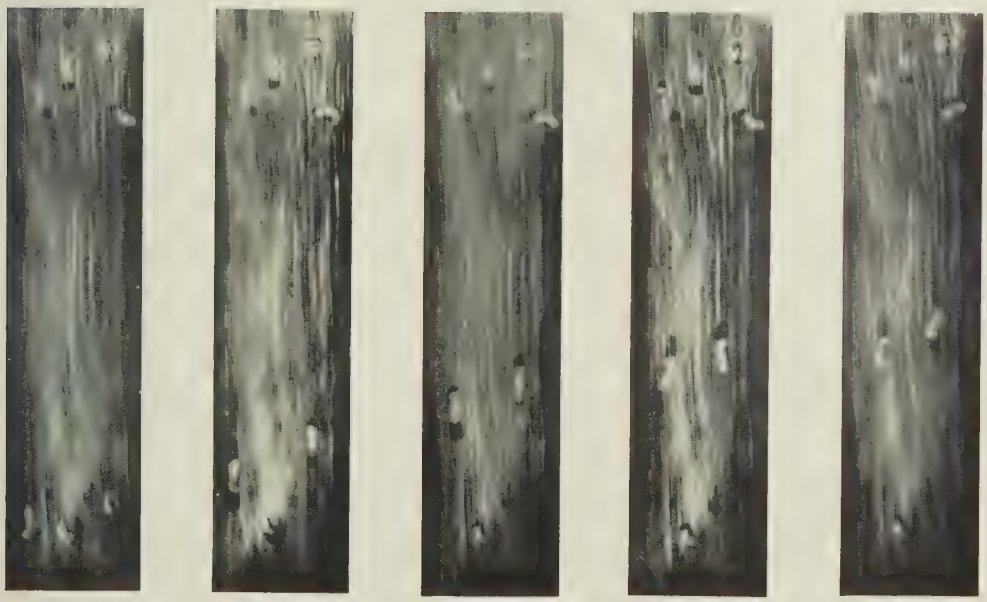


Fig. 1.



a *b* *c* *d* *e*

Fig. 2.

THE TOXICITY OF SULPHUR DIOXIDE TO THE BED-BUG *CIMEX LECTULARIUS* L.

By H. C. GOUGH

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(With 3 Text-figures)

1. Introduction

Sulphur dioxide, usually obtained by burning sulphur, when used as a fumigant for bug infested houses, has not always been successful (McKenny Hughes, 1935). The purpose of this work was to attempt to explain the occasional failures of sulphur dioxide and if possible to suggest how its action might be improved.

2. Previous work

Previous work on the toxicity of sulphur dioxide to the bed-bug has been very indefinite. The fumigation chamber employed was usually an ordinary room in which the concentration would be falling throughout the fumigation. Even when the concentration was measured, it was usually at one unspecified time and place, and this is not necessarily a reliable indication of the concentration actually attained in the crevices where the bugs are likely to be. Some workers have attempted to simulate the protection normally afforded to the bugs by their surroundings, by covering the experimental insects in an arbitrary number of wrappings. The temperature and humidity at the time of the fumigation was rarely stated. When burning sulphur is used, the suggested amount varies from 2 lb. sulphur/1000 cu. ft. for 6 hr. (Marlatt, 1916) to 6 lb. sulphur/1000 cu. ft. for an unstated period, presumably overnight (McKenny Hughes, 1935). It is sometimes recommended that this process be repeated after an interval of about 3 weeks, to destroy the nymphs which have emerged from eggs which survived the first fumigation (McDaniel, 1926; Gunn, 1933; McKenny Hughes, 1935). Gunn (1933) suggests that as the penetration of the fumigant would be slow into the cracks and crevices in which the eggs are usually laid, the eggs would be likely to escape unharmed, whatever the fumigant. It is to be presumed, however, that the eggs would be laid in the same places where the bugs were concealed and that this factor of slow penetration would be equal for all stages. Fetscher (1927) has recorded adults surviving at concentrations which killed the eggs. His experimental insects were, however, surrounded by layers of paper to simulate the natural shelter of the bugs. Schlupp (1916) considered that the eggs of the bed-bug were more resistant than other stages to sulphur dioxide, and this fumigant is generally regarded as a poor ovicide. Schiemann (1918), Imes (1926) and others have found that eggs of lice were more resistant than adults to sulphur dioxide.

When sulphur is burnt, about 10% of sulphur trioxide is formed (Lubatti, 1936). Whether the physical state of this substance, which is in the form of a fog, would permit very much of it to enter the more remote hiding places of the bugs is doubtful.

Liquid sulphur dioxide liberated from small cylinders is also used. Gunn (1933) recommends 60–80 fl. oz. (equivalent to about 90–120 oz. by weight) per 1000 cu. ft.

It was therefore decided to commence this work with a survey of the relative resistance of the different stages of the bed-bug to sulphur dioxide under constant conditions and later to consider the effect of other factors.

3. The apparatus

The apparatus was designed by Dr H. H. S. Bovingdon and is a modification of that described by him (Bovingdon, 1934). It has been described in detail by Gough (1939). Briefly, temperature and humidity were controlled and the gas was circulated through the insect chamber throughout each test. The sulphur dioxide was obtained from a glass syphon and the approximate amount required in each test, was measured out as a gas by volume. This was admitted to the evacuated apparatus and the pressure restored to atmospheric, with air of 60 % relative humidity. A sample of the gas mixture was taken at the beginning and end of each test and absorbed in 5 c.c. of $N/10$ sodium hydroxide containing 5 % glycerol, and titrated with $N/100$ iodine solution, using starch solution as indicator (Lubatti, 1936). The accuracy of this determination was from $\pm 0{\cdot}4$ to $\pm 2{\cdot}0$ % depending on the concentration. The concentration of the gas in mg./litre could be calculated from the result of this titration. A small amount of gas was absorbed during each test by stopcock grease, the bugs and their container, but usually this loss did not exceed 1 %.

4. Technique of breeding the bed-bugs

The bugs were kept in desiccators at 60 % relative humidity in an incubator at 23° C. and were fed once a week on a rabbit. In each tube of bugs was a piece of blotting paper to which the bugs clung and on which the eggs were laid. This paper was removed every 2 days from cultures of adults and the eggs on it could be fumigated at any desired age. When required for tests, the eggs were brushed off the paper into a small muslin bag. This operation, when carefully done, did not increase the mortality amongst the controls, which was usually between 5 and 10 %. In some tests, in which eggs were exposed for periods of 6 hr. or over, it was found that the vapour of the mercury in the apparatus was highly toxic. This and other references to the toxicity of mercury vapour to insects have been noted by Gough (1938). At the exposures of $2\frac{1}{2}$ hr. used in all the tests described in the present paper the mercury vapour did not appear to exert any very noticeable toxic effect. Those eggs which were not used for fumigation tests were allowed to hatch and the young nymphs given an opportunity of feeding after 2 days. The few that did not feed were either destroyed or transferred to the next lot of nymphs to be fed. Nearly all those that did feed, moulted into the 2nd instar about 5 days later and were fed again on the 7th day. Once again, those that had not fed, and also those which had failed to moult after the previous meal were removed. This process was repeated until the final moult had taken place. After this, the newly moulted adult bugs did not feed readily for several days. They received their first meal as adults, therefore, about a week after moulting. After this meal they were sorted into males and females, and cultures containing ten of each sex were made up. Large numbers of bugs of any age or stage were therefore available when required.

The bugs for each test were placed in a muslin bag and introduced into the insect chamber about 20 min. before the gas was introduced. Usually, but not always, a similar lot was used as a control.

5. Effect of sulphur dioxide and criterion of death

After each test the insects or eggs were transferred to the incubator where they had been previously kept. The following day they were counted and they were kept under observation for about a further 10 days. Usually the day after the test most of the bugs were either obviously alive or dead. A few remained moribund for several days and most of these ultimately died. There was no mortality amongst the control nymphs or adults. Of the nymphs that had been fed 2 or 3 days before fumigation, the gut of nearly all the dead insects had burst and the blood of the last meal was visible throughout the body. A similar effect

has been observed for ethylene oxide by Mayer (1934) and others. This bursting of the gut was always fatal and had not occurred in those insects which were moribund. This might explain the difference in resistance between starved and fed bugs (see § 7).

After tests on eggs, the young nymphs were removed and counted as they hatched. They appeared normal and when given the opportunity, nearly all fed and moulted. This was also true of other stages of bugs which survived fumigation.

6. The relative resistance of the different stages

Eggs were fumigated at 0–2, 2–4, 4–6, and 6–8 days old. At 23° C. they hatch about the 9th day. Tests were carried out on all instars 2 days after feeding. Only young adults were used and these were fumigated 2 days after their first meal. The results of tests on males and females in the adult stage were recorded separately, but as there was apparently no difference in resistance between the two sexes, the results were combined. The numbers of insects used in each test varied from 60 to 100 and the average numbers for tests on each stage are given in Table 1. The bugs or their eggs were exposed to various known concentrations of sulphur dioxide for $2\frac{1}{2}$ hr. at 23° C. and 60% relative humidity and the number of survivors recorded. The concentration at and above which there were no survivors, was taken as approximately the region of complete mortality. In this region the concentrations at each test were separated by not more than 0·02 mg./l., usually only by 0·01 mg./l. About 10 tests were performed on each stage (see Table 1).

Table 1. *Mg. l. of sulphur dioxide necessary to kill various stages of the bed-bug at 23° C., 60% relative humidity, for $2\frac{1}{2}$ hr. exposure*

Stage	No. of tests	Average number of insects used in each test	Mg./l. of SO_2 to give 100% kill
0–2 day old eggs	11	84	16·7
2–4 ,, ,, ,,	7	104	15·8
4–6 ,, ,, ,,	15	71	12·0
6–8 ,, ,, ,,	12	63	8·9
1st instar nymphs unfed 1 day after hatching	8	102	6·2
1st instar nymphs 2 days after feeding	9	82	6·1
2nd instar nymphs 2 days after feeding	7	83	6·0
3rd instar nymphs 2 days after feeding	12	65	5·7
4th instar nymphs 2 days after feeding	10	56	5·8
5th instar nymphs 2 days after feeding	10	57	6·1
Adults 2 days after first meal	10	61	4·2

It is clear that, especially during the first half of their incubation period, eggs are more resistant than any other stage and that this resistance decreases as the time of hatching approaches. It is interesting to note that Bovingdon (1935) for hydrogen cyanide, and Mayer (1934) for ethylene oxide, found that the eggs of the bed-bug were less resistant than any other stage. Both also found that adults were less resistant to these fumigants than nymphs. Peters (1936) records this same order of resistance to ethylene oxide and hydrogen cyanide,

i.e. nymphs (most resistant) adults, eggs. Gunderson & Strand (1939) have also confirmed this but found that to chloropicrin the eggs were more resistant than the later stages. Thus, it appears that to sulphur dioxide and chloropicrin the eggs of the bed-bug are more resistant than any other stage, whereas to hydrogen cyanide and ethylene oxide they are less resistant than any other stage. Similar results have been found by various workers for other insects and are referred to by Gough (1939).

The slight differences shown in Table 1 in the resistance of the different nymphal instars to sulphur dioxide cannot be considered significant.

7. The effect of starvation on resistance

Bed-bugs withstand starvation for long periods. Fetscher (1927) found that starved adult bugs were more resistant to hydrogen cyanide than bugs which had been recently fed. Mayer (1934) found no difference in the concentrations of ethylene oxide necessary to give complete kills of starved and fed nymphs of the bed-bug but the concentration necessary to give about 25% kill of starved nymphs was, however, higher than that for recently fed nymphs. Since the present work was carried out, Busvine (1938) has shown that while the resistance of 4th and 5th nymphal instars to hydrogen cyanide decreases with starvation, their resistance to ethylene oxide increases with starvation. It thus appeared important to compare the resistance of fed and starved bugs to sulphur dioxide. Experiments were accordingly carried out on 1st and 5th instar nymphs and adults. There is a difficulty in that the nymph of the bed-bug moults a fixed number of days after each meal. Thus, to compare the resistance of 5th instar nymphs which have been fed recently, with the resistance of nymphs which have been starved for some time, the period of starvation of the latter would have to commence from the meal in the 4th instar. For convenience it is reckoned from the time of moulting into the 5th instar which would occur about 6 days after that meal. This is why starved 2nd instar nymphs are compared with recently fed 1st instar nymphs.

Nearly 200 individuals of 1st and 2nd instar nymphs were used in each test and about 60–70 individuals of 5th instar nymphs and adults. As before, all tests were carried out at 23° C. and 60% relative humidity, for exposures of $2\frac{1}{2}$ hr.

Separate tests were performed on 1st instar nymphs fumigated on the day of feeding and the same fumigated 2 days after feeding. As the two lots showed no difference in resistance the results were combined. No difference in resistance between the sexes was observed and the results of tests on males and females were combined. There was no mortality amongst the controls. About a third of the adult females that were starved died during the second half of their starvation period of one month although those females which survived were not less resistant to sulphur dioxide than the males. Johnson (1937) records mated female bugs, when fed on various hosts, as living after one meal for mean periods of 53–112 days. There were, however, several differences in the conditions under which Johnson's bugs, and those of the present author were kept. Mellanby (1938) has drawn attention to the effect of activity on the resistance of bed-bugs to starvation. It is possible that when the eggs were removed in order that the culture should not be overrun with young nymphs, the disturbance of the bugs by causing great activity was responsible for their high mortality during starvation.

For the study of the effect of starvation on resistance the whole range of the dosage-

mortality curve was explored and the results treated by Bliss's method (1935*a*). The percentages were calculated to the nearest 1 % and when converted to probits, to the first three significant figures. The concentrations were accurate to 0·05 mg./l. of sulphur dioxide and the first three significant figures of the logarithm of the concentration were used. The 50 % kills with their limits of error were calculated from Bliss's formula 28 (Bliss, 1935*b*) (the statistic "t" in this formula was read at a value of $P = 0{\cdot}05$) and are given in Table 2. The graphs are shown in Figs. 1–3. The approximate concentrations corresponding to 99·9 % kill or about 8·1 probits have been extrapolated from the regression lines and are also shown

TABLE 2. *Mg./l. of sulphur dioxide necessary to give* 50 % *and* 99·9 % *kill to starved and fed bed-bugs of various stages.*

	Conc. of SO_2 to give 50 % kill mg./l.	Conc. to give 99·9 % kill (extrapolated) mg./l.	Conc. to give 100 % kill (from Table 1) mg./l.
1st instar nymphs:			
0 and 2 days after feeding	3·81 ± 0·05	5·5	6·1
2 days after feeding on human blood	3·66 ± 0·12	—	—
4 days after feeding	3·36 ± 0·04	4·7	—
2nd instar nymphs:			
14 days after moulting (unfed)	4·08 ± 0·15	8·9	—
5th instar nymphs:			
2 days after feeding	4·60 ± 0·06	6·8	6·1
1 day after moulting (unfed)	3·01 ± 0·12	5·8	—
14 days after moulting (unfed)	5·30 ± 0·05	6·3	—
49 days after moulting (unfed)	5·58 ± 0·21	12	—
Adults:			
2 days after 3rd meal	2·47 ± 0·08	3·9	—
28 days after 3rd meal	3·12 ± 0·18	6·8	—
2 days after 1st meal	—	—	4·2

in Table 2. Where available, the concentrations necessary to give a complete kill, as determined by the method used in the first part of this work, are also shown in Table 2 in order that they might be compared with the extrapolated value. At the concentration to give 99·9 % kill of 1st instar nymphs, as calculated by Bliss's method, several survivors were actually recorded, but it must be remembered that the errors of computation in this region of the regression line are very great. For 5th instar nymphs and adults Bliss's method gave a slightly higher figure. On the whole, however, the two methods compare remarkably well.

These results must only be regarded as preliminary. As it was thought that the effect of disturbance of the bugs during transference from their tubes to the muslin bags in which they were fumigated was very great and probably had a greater effect on the starved bugs, it was decided not to continue these experiments until an apparatus with larger insect chambers into which the whole tube of bugs could be placed, was available.

The data as presented, do not rule out the possibility that differences in resistance with starvation are due to differences of age. Such a possibility could be investigated by comparing the resistance of bugs which had been starved for some time with the resistance of bugs of the same stage which had been starved an equal length of time and then given a meal.

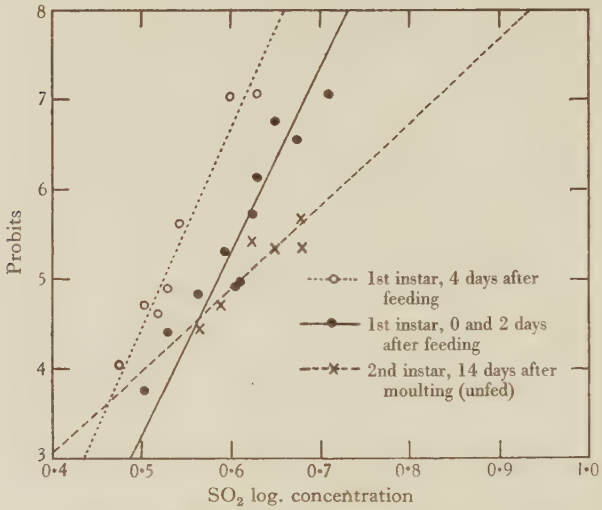

Fig. 1. Dosage-mortality regression lines (Bliss's method) of bed-bugs in 1st and 2nd nymphal instars exposed to sulphur dioxide for 2½ hours at 23° C. and 60 % relative humidity.

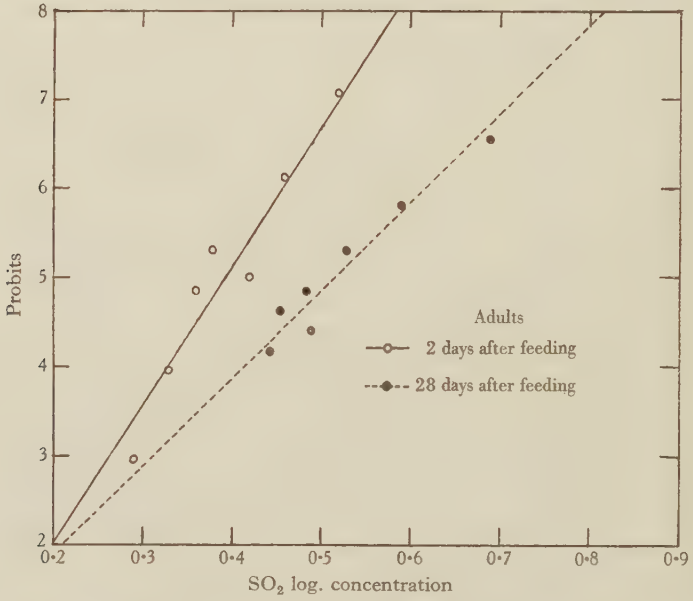

Fig. 2. Dosage-mortality regression lines (Bliss's method) for adult bed-bugs exposed to sulphur dioxide for 2½ hours at 23° C. and 60 % relative humidity.

It seems very likely, however, that the resistance of nymphs and adults does increase with starvation. For 1st instar nymphs, the resistance appears to decrease slightly in the period between feeding and moulting. A few tests on 5th instar nymphs, not recorded here, indicated a similar decrease. 5th instar nymphs immediately after moulting were more susceptible than those in any other condition tested and the resistance of these and 1st instar nymphs increased after moulting with continued starvation. While there was little difference in the concentrations necessary to give 50 % kills of 5th instar nymphs starved for 14 and 49 days after moulting, the variation among those starved for the longer period was so great that there was a considerable difference between the extrapolated concentrations necessary to give 99·9 % kill. In fact, for the nymphs starved for 49 days, this concentration approaches that necessary to give complete kills of eggs at their more resistant stages. For starved individuals of both 2nd instar nymphs and adults there was a considerably greater variation, with a lessening of the slope of the regression line, than among recently fed bugs.

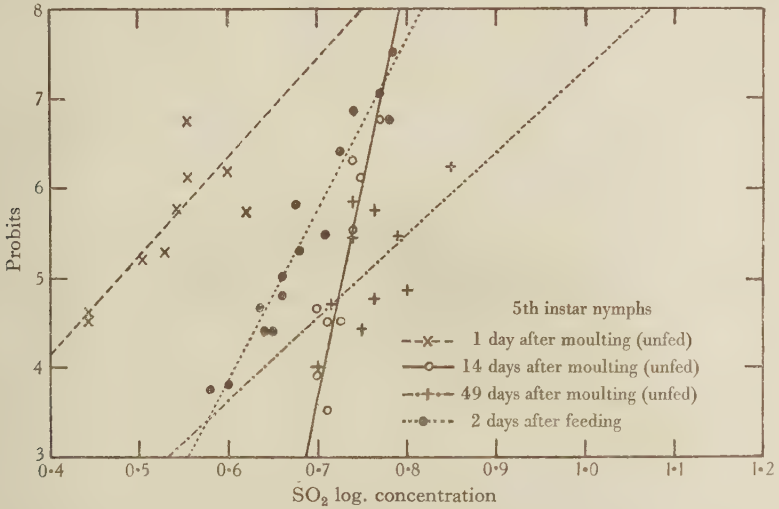

Fig. 3. Dosage-mortality regression lines (Bliss's method) of bed-bugs in the 5th instar exposed to sulphur dioxide for 2½ hours at 23° C. and 60 % relative humidity.

A few tests were carried out on 1st instar nymphs fed on human blood (see Table 2). These nymphs were barely significantly less resistant than the nymphs fed, as was usual, on rabbits' blood. This experiment cannot be considered as having any great significance as only one individual of each host and only one stage of the bed-bug was used.

8. Discussion

From the available data it is difficult to say definitely whether eggs are much more resistant than nymphs which have been starved for some weeks. If they are, it might account for the occasional failure of sulphur dioxide fumigations where only one fumigation was carried out. It must be pointed out that the word "failure" may have two interpretations:

(1) That live nymphs or adults are observed a day or so after the fumigation. Unless these

have left the house during the fumigation and later returned, it may be regarded as almost certain that large numbers of eggs and possibly some nymphs have escaped destruction.

(2) That bugs were observed, or their presence detected, a few months after the fumigation. These may have been introduced from another source since the fumigation, or they ma have been derived from eggs which survived the fumigation.

Whether the apparently high resistance of starved nymphs is of great importance or no depends to some extent on whether such long periods of starvation occur normally whe food is available for the bug.

It must be emphasized that further work on the effect of starvation on the resistanc of bed-bugs to sulphur dioxide is necessary and the conclusions drawn here can only b regarded as tentative.

9. Summary

1. The egg of the bed-bug is more resistant to sulphur dioxide than any other stage. Th resistance is greatest during the first half of the incubation period and then graduall decreases. Nymphs are more resistant than adults.

2. Starved nymphs and adults appear to be more resistant than recently fed ones. Fro preliminary experiments it appears that the resistance of nymphs starved for long perio may be nearly as high as that of eggs.

The work was carried out at the Imperial College of Science, Biological Field Statio Slough, on behalf of the Committee on Bed-bug Infestation of the Medical Research Counci Thanks are due to Professor J. W. Munro, under whose direction the work was done, and t Dr A. B. P. Page for much helpful advice.

REFERENCES

Bliss, C. I. (1935*a*). The calculation of the dosage-mortality curve. *Ann. appl. Biol.* **22**, 134.

—— (1935*b*). The comparison of dosage-mortality data. *Ann. appl. Biol.* **22**, 307.

Bovingdon, H. H. S. (1934). An improved laboratory fumigation apparatus. *Ann. appl. Biol.* **21**, 70

—— (1935). The bed-bug (*Cimex lectularius* L.) Fam. Cimicidae. *Rochester Nat.* N.S., **1**, 3.

Busvine, J. R. (1938). The toxicity of ethylene oxide to *Calandra oryzae*, *C. granaria*, *Tribolium castaneum* and *Cimex lectularius*. *Ann. appl. Biol.* **25**, 605.

Fetscher, R. (1927). Über die Bekämpfung der Bettwanze mit Blausaüre (Zyklon B) und Schwefeldioxyd. *Reichsgesundheitsblatt*, **11**, 228; **12**, 253.

Gough, H. C. (1938). Toxicity of mercury vapour to insects. *Nature, Lond.*, **141**, 922.

—— (1939). Factors affecting the resistance of the flour beetle *Tribolium confusum* Duv. to hydrogen cyanide. *Ann. appl. Biol.* **26**.

Gunderson, H. & Strand, A. L. (1939). Toxicity of hydrogen cyanide, chloropicrin, and ethylene oxide to eggs, nymphs and adults of the bed-bug. *J. econ. Ent.* **32**, 106.

Gunn, W. C. (1933). The bed-bug (*Cimex lectularius*). Prevention of house infestation. *Rep. Dep. Hlth Scot.* no. 2.

Imes, M. (1926). Lice, mange, and ticks of horses and methods of control and eradication. *Farmers' Bull. U.S. Dep. Agric.* no. 1493.

Johnson, C. G. (1937). The relative values of man, mouse and domestic fowl as experimental hosts for the bed-bug, *Cimex lectularius* L. *Proc. zool. Soc. Lond.* A, **107**, 107.

Lubatti, O. F. (1936). Determination of fumigants. VII. Determination of sulphur dioxide and sulphur trioxide from burning sulphur. *J. Soc. chem. Ind., Lond.*, **55**, 344.

MARLATT, C. L. (1916). The bed-bug. *Fmrs' Bull. U.S. Dep. Agric.* no. 754.
MAYER, K. (1934). Die letale Dosis Äthylenoxyd bei *Calandra granaria, Tribolium confusum* und *Cimex lectularius*. *Arb. physiol. angew. Ent. Berl.* **1**, 257.
MCDANIEL, E. (1926). Fleas and bed-bugs. *Circ. Bull. Mich. Agric. Exp. Sta.* no. 94.
MCKENNY HUGHES, A. W. (1935). The bed-bug as a housing problem. *J. roy. San. Inst.* **55**, no. 9.
MELLANBY, K. (1938). Activity and insect survival. *Nature, Lond.*, **141**, 554.
PETERS, G. (1936). Chemie und Toxikologie der Schädlingsbekämpfung. *Samml. chem. Vortr.* **31**, 59.
SCHIEMANN, O. (1918). Ueber schwefelige Saüre als Mittel zur Tötung von Laüsen und Flöhen. *Z. Hyg. Infektkr.* **78**, 389.
SCHLUPP, W. F. (1916). Fumigation with sulphur. *J. Dep. Agric. S. Afr.* **4**, 132.

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SOME PRELIMINARY EXPERIMENTS WITH β-BUTOXY-β′-THIOCYANODIETHYLETHER AS AN INDUSTRIAL INSECTICIDE

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INTRODUCTION

THIS paper gives the results of some preliminary experiments on the biological action of β-butoxy-β′-thiocyanodiethylether, one of the organic aliphatic thiocyanates. The work has been done on some of the more important indoor insect pests.

Apparently only two other organic thiocyanates have shown promise as commercial insecticides at the present time. They are dodecyl or lauryl thiocyanate, which has been studied by Bousquet *et al.* (1935) and by Kearns & Martin (1935), and α-naphthyl isothiocyanate which has recently been reported on by Tischler and his co-workers (1938). Experiments with other thiocyanates are described by Wilcoxon & Hartzell (1935). There is little published work on the use of β-butoxy-β′-thiocyanodiethylether against indoor pests. Some work has recently been described by Badertscher (1936), who conducted tests by two methods with various insecticides against house-flies, and Woodbury (1938) more recently has described some tests in which the German cockroach was used.

Besides the experiments described in this paper field trials and more detailed laboratory experiments have been carried out and will be reported in later publications.

INSECTICIDE MATERIALS

β-butoxy-β′-thiocyanodiethylether is also called normal butyl carbitol thiocyanate (or rhodanate). It is sold under the proprietary name of Lethane. The concentrated thiocyanate is obtainable in a refined kerosene at two dilutions, Lethane 384 which contains 50 % thiocyanate and Lethane 410 which contains 75 %. Throughout the paper the terms "thiocyanate" and "carbitol thiocyanate" have been used as abbreviations for β-butoxy-β′-thiocyanodiethylether.

Two types of oil were used for making the working dilutions, a highly refined odourless kerosene called odourless distillate and a highly refined white oil which is nearly odourless and tasteless and is marketed as Shell oil 24210. The following are the specifications of the two oils:

	Shell oil 24210	Odourless distillate
Sp. gr.	0·862	0·779
Flashpoint closed	320° F.	158° F.
Flashpoint open	335° F.	—
Visc. Redw. I at 70° F.	118 sec.	—
Visc. Redw. I at 60° F.	—	32 sec.
Initial boiling-point	—	200° C.
Final boiling-point	—	270° C.
Unsulphonatable residue	99·2 %	—

In most of the experiments where shell oil 24210 was the diluent, the 75 % thiocyanate was used.

In some experiments dodecyl rhodanate was used. The concentrated material was a liquid containing nearly 100 % rhodanate, but with some unknown impurities present.

Insect material

Owing to limitations of space and labour it was only possible to breed one insect under standard conditions, the bed-bug *Cimex lectularius* L., which was reared at 23° C. and approximately 70% relative humidity. Apart from this species, some insects were bred in the laboratory, and we are indebted to Dr G. Mansbridge of Imperial Chemical Industries for supplying us with others.

A number of difficulties were encountered in breeding. Some stocks of *Plodia interpunctella* Hb. became severely infested with *Mattesia dispora* (Musgrave & Mackinnon, 1938). At one stage the stock of *Ephestia kühniella* Z. appeared unhealthy and was suspected of bacterial disease. In spite of the standard conditions of breeding a marked variation in the susceptibility of the stocks of *Cimex lectularius* L. was also found during the course of the work. As far as possible these factors were taken into account in setting out the results, and a considerable number of results were discarded because the resistance of the insects was considered to be below normal or because we were dissatisfied with our technique at the time.

Methods of treatment

Three methods were used:

(*a*) The insects were sprayed directly.

(*b*) The fumigant effect of the insecticides was tested.

(*c*) The effect of films of the insecticides was tested.

Methods (*b*) and (*c*) give information on the residual effect of the insecticide.

All the experiments were exploratory and can be regarded only as guides for more detailed work.

(*a*) *The spraying method*

The spraying was done in a specially constructed spraying tower. This apparatus gives an even deposit over a 6 in. circular glass plate. It was used because the smaller area covered by the Tattersfield apparatus, which we consider to be the only satisfactory alternative, was not sufficient for many of the active insects used in these tests.

A full account of the spraying tower will be published later by one of us (Potter). In essentials it consists of an atomizing gun spraying through a tubular structure on to a glass plate resting on a brass foundation, which may be easily inserted and removed. The atomizing gun is worked by compressed air from an air cylinder or from a compressor, the air passing through an air filter and adjustable reducing valve. The weight of insecticide deposited on the plate depends partly on the rate of flow of the insecticide, which can be adjusted by a needle valve in the atomizing gun, and partly on the air pressure and the physical properties of the insecticide. For each series of sprayings the time taken by the insecticide to flow out was checked and air flow for each spraying was checked by means of a pressure gauge and an air-flow meter.

Some variations in the technique were tried in the initial experiments. The final procedure was as follows. Insects obtained from cultures were placed in glass tubes or Petri dishes and from these they were picked or tapped on to the basal plate of the spraying tower. The plate was then inserted into the tower and the insects were sprayed with the measured amount of insecticide, the time taken for the insecticide to pass through the gun being noted with a stop watch. The air-pressure and air-flow readings were observed and kept constant over any given series of experiments. After spraying, the insects were usually left in the tower for a short time, usually 1 min., but this interval is not necessary. The basal plate was then detached and the insects removed. Insects that had crawled off the glass plate on to the surrounding brass were discarded. Control experiments were made with each batch of insects; some were sprayed with the diluent carrier only, and some were not sprayed but were handled in the same way as the sprayed insects as far as possible. Forceps used to pick up the insects were cleaned in alcohol after each lot of insects had been removed from the glass plate. After each spraying the basal plate and washer were removed and cleaned and a clean glass plate and washer used for the next spraying. The tower and gun were cleaned when necessary by spraying through with xylol and acetone.

Explanation of terms used in Tables 1–5. In these tables the following terms are used: 24210 = Shell oil 24210, O.D. = odourless distillate, dodecyl = dodecyl rhodanate, and thiocyanate = β-butoxy-β'-thiocyanodiethylether. The weight of spray deposited is given in g./sq. cm. This weight was determined by weighing glass slips of known area, in glass weighing bottles, before and after spraying under the same conditions as those for the given experiment. The weights given are only approximate,

because some variation takes place. The exact amount of this variation has not yet been determined accurately, but the data so far available indicates that it is within 10%.

Recent work suggests that on different days some weeks apart the variation in weight deposited even under similar conditions is somewhat variable.

Results of the Spraying Experiments.

A preliminary experiment was made on the adults of *Tribolium castaneum* Hbst. with 5% thiocyanate in shell oil 24210, using a spray deposit of approximately 0·001 g./sq. cm. a kill of 94% was obtained, the oil alone killed 32%, unsprayed insects showed a mortality of 13%, 349 insects were used in this test.

Table 1 shows the results of spraying the eggs of *Cimex lectularius* L. with the carbitol thiocyanate in two different carriers. The weights given are from early experiments. The eggs were sprayed on the paper on which they had been laid.

Table 2 sets out the results of an experiment on fully grown larvae of *Plodia interpunctella* Hb. The percentage kills shown are the average of two series which agreed fairly well except when 3% thiocyanate was used. After spraying, these insects were transferred to dishes containing blotting paper so that they were able to remove much insecticide from their bodies. This factor, as would be expected, has an effect on the kill obtained as is well shown in Table 3.

Table 3 shows the results of spraying fully grown larvae of *Ephestia kühniella* Z. with carbitol thiocyanate in two different carriers. It also shows the effect of the presence or absence of paper in the dishes to which the insects were transferred after treatment. The control in Exp. 11 gave a 14% kill indicating that perhaps the unexpectedly high kill in Exp. 9 was partly due to an unusual sensitiveness of this batch of insects.

Table 4 gives the results of spraying eggs of the bed-bug, *Cimex lectularius* L., on the paper on which they were laid, with various concentrations of carbitol thiocyanate in odourless distillate. (Bugs were bred in small glass tubes containing blotting paper and covered with muslin held in position by adhesive tape; they were able to feed through the meshes of the muslin. A great number of the eggs were laid on the blotting paper. This was removed as required and the eggs were scraped off one side of the paper. It was found to be better to spray the eggs on the paper as scraping them off undoubtedly injured some of them.) Some variation was shown by the duplicate tests at each concentration, which may be due to biological causes or to physical causes due to the apparatus or to both, but in spite of this, it is evident that the thiocyanate has good ovicidal properties.

There were two other experiments in which two lots of eggs were divided into a number of batches. Each batch was sprayed with a constant quantity of odourless distillate under conditions known to give a constant dosage over a period of, at least, 1 day. The batches from one lot, aged 3–5 days, were all sprayed on one day, those of the other lot, aged 5–8 days, on another day. The kills obtained in the first lot were 29, 50, 33, 53 and 29% for the various batches. In the second lot the kills in the separate batches were 40, 70 and 52%. We have good evidence for thinking that the doses received by the different batches in the two lots were the same; though the batches in one lot did not necessarily get exactly the same dose as those in the other lot. The variation in kill obtained suggests that there is some variation in the biological material. As the experiments set forth in Table 5 were done over a period of some weeks and as there was therefore probably some variation in the dosage, the variation in the results shown in this table would seem to be due to both causes. This matter will be discussed in a later paper.

TABLE 1. *Effect of the thiocyanate on the eggs of* Cimex lectularius *L.*

Thiocyanate %	Diluent	Approx. wt. of spray deposited in g./sq. cm.	No. of insects used	% kill
2½	O.D.	0·0006	113	97
None	O.D.	0·0006	80	64
None	None	—	71	3
3	24210	0·001	375	99·6*
None	None	—	142	25

* Average of three tests.

TABLE 2. *Effect of different concentrations of the thiocyanate in Shell oil* 24210 *on the fully grown larvae of* Plodia interpunctella *Hb. Approximate weight of spray deposit* 0·001 *g./sq. cm.*

Thiocyanate %	No. of insects used	% kill
0	44	16
0	32	20
2	19	23·5
3	36	78
3½	21	76
4	20	70
4½	41	86

Note. In this experiment the insects were transferred after spraying to dishes containing blotting paper.

TABLE 3. *Effect of* 3 % *thiocyanate in two different carriers on the larvae of* Ephestia kühniella *Z. The effect of the after treatment, which is described in the last column and indicates whether the insects were transferred after spraying to dishes containing semi-glazed paper or not, is also shown*

Diluent	Approx. wt. of spray deposited in g./sq. cm.	No. of insects sprayed	% kill	After treatment
24210	0·001	19	95	No paper
24210	0·001	22	27	Paper
Control	—	22	9	Paper
Control	—	20	0	No paper
O.D.	0·0008	22	96	No paper
O.D.	0·0008	24	25	Paper
Control	—	1	0	No paper
Control	—	13	8	Paper
O.D.	0·0001	26	96	Paper
O.D.	0·001	22	100	No paper
Control	—	21	14	No paper

TABLE 4. *Effect of spraying eggs of* Cimex lectularius *L. with varying concentrations of the thiocyanate in odourless distillate. Approximate weight of spray deposit* 0·001 *g. sq. in.*

	Eggs aged 2–6 days		
Thiocyanate %	No. of eggs used	No. of tests	Mean % kill
1/16	283	5	65
1/8	470	6	65
1/4	305	5	92
3/8	157	3	98
1/2	209	5	96
O.D. Control	501	7	75
Unsprayed control	486	7	7

TABLE 5. *Results of experiments in desiccators on the comparison of the vapour effect of the thiocyanate with various other substances*

Species of insect ...	*Sitophilus oryzae* L.		*Sitophilus oryzae* L.		*Sitophilus granarius* L.		*Ephestia kühniella* Z.		*Tenebrio molitor* L.		*Cimex lectularius* L.		*Cimex lectularius* L.		*Cimex lectularius* L.		*Cimex lectularius* L.	
Stage of insect ...	Adult		Adult		Adult		Larva		Larva		Egg 2–5 days old		Egg		Egg 3–5 days old		Egg	
Time of exposure (hr.) ...	$23\frac{1}{2}$		$28\frac{3}{4}$		24		$26\frac{1}{4}$		17		$5\frac{1}{2}$		$21\frac{1}{3}$		23		$16\frac{2}{3}$	
Age of liquid in desiccator (days) ...	7		2		12		5		—		1		35		—		14	
Temp. (° C.) (approx. average) ...	23		21		23		16		—		—		16		15		19	
Liquids in desiccators	% kill	No. of insects used	% kill	No. of insects used	% kill	No. of insects used	% kill	No. of insects used	% kill	No. of insects used	% kill	No. of insects used	% kill	No. of insects used	% kill	No. of insects used	% kill	No. of insects used
75 % thiocyanate in O.D.	—	—	—	—	—	—	8·7	48	0	9	—	—	100	15	100	99	100	66
50 % thiocyanate in O.D.	98·6	71	100	107	65·5	5·5	80·8	52	0	9	100	133	100	12	—	—	100	32
0·17 % pyrethrins in O.D.	—	—	—	—	—	—	—	—	0	9	—	—	—	—	—	—	—	—
O.D.	8·6	58	9·7	113	—	—	—	—	0	9	—	—	8·2	49	—	—	5·3	19
100 % dodecyl rhodanate	—	—	—	—	—	—	1·9	53	—	—	—	—	—	—	12·05	83	—	—
50 % dodecyl in O.D.	15·6	64	6·1	98	14·6	48	—	—	—	—	—	—	—	—	—	—	—	—
5 % thiocyanate in 24210	—	—	—	—	—	—	7·8	51	—	—	—	—	—	—	100	80	—	—
0·3 % pyrethrins in 24210	—	—	—	—	—	—	5·6	54	—	—	—	—	—	—	9·8	112	—	—
1 % pyrethrins in O.D.	34·4	64	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
24210	—	—	—	—	—	—	2·0	50	—	—	—	—	—	—	.	.	.	.
3 % thiocyanate in O.D.	47·8	67	53·3	92	—	—	—	—	—	—	—	—	—	—	—	—	—	—
3 % thiocyanate in O.D. scented	50	62	35·7	115	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Carbon disulphide	—	—	—	—	—	—	—	—	—	—	100	155	.	.	.	.	—	
Mercury	31·8	63	0	109	—	—	—	—	—	—	16·7	114	—	—	—		.	—
Formalin	100	65	100	94	.	—	—	.	—	—	—	—	—	—	—	—	—	—
Nothing	17·7	62	15·5	97	11·5	52	10·4	48	0	9	5·3	171	10·9	37	9	100	6·9	2·9

(*b*) *Experiments on the toxicity of the vapour phase*

Description of experiments. Insects in glass tubes (3 × 1 in.) covered with muslin were put into the upper part of chemical desiccators containing the insecticides to be tested in the lower compartment: 10 ml. of toxic substance were used in all the experiments. The upper and lower compartment of the desiccators were separated by a glass plate which was arranged to allow diffusion of gases between them.

In most of the experiments desiccators, 10 in., were employed, but in some instances, one larger desiccator was used for the control, for the insecticide diluents or for the carbitol thiocyanate solution.

It was assumed that air in the desiccators was saturated with the vapour of the liquid placed in the lower compartment. There was no control over the dosage except that specimens could be left in the desiccators for various lengths of time. It was important that a desiccator should not be used too often without changing the liquid.

Two types of experiments were conducted. In one type, batches of insects were sorted into muslin-topped tubes, each of which was put in a separate desiccator, containing a solution under test. One tube was always put in a control desiccator which contained air only. The insects were then subjected to the action of the vapours for a known time. These experiments showed that carbitol thiocyanate was definitely toxic in its vapour phase to some insects and particularly to the eggs of the bed-bug *Cimex lectularius* L. The larvae of *Tenebrio molitor* L. were not affected by the vapour. The grain and rice weevils appeared to be fairly susceptible.

In the second type, several tubes each containing insects were put into one desiccator containing an insecticide liquid and were left for various lengths of time. When the last tube was removed a control tube was removed from a desiccator containing air only. These experiments indicated that the eggs of *Cimex lectularius* L. were particularly susceptible to the vapour; they appeared to be more susceptible than the adults. In one instance, an exposure for $3\frac{1}{2}$ hr. to the vapour from a 50% carbitol thiocyanate solution in odourless distillate was sufficient to give a 100% kill with 106 eggs aged 1–4 days. The control for the experiment showed a mortality of approximately 8% with 74 eggs. The adults of the rice weevil appeared to be fairly susceptible but adults of the beetle *Dermestes vulpinus* seemed to be only slightly affected after an exposure of several hours.

An experiment was conducted with eggs of *Cimex lectularius* in which the same lot of eggs was divided into several batches some of which were placed in one desiccator over the thiocyanate and some in another containing a similar solution. The results from the two desiccators were quite different, which may have been due to the fact that the desiccators had been used to a different extent, and the experiment emphasizes the crudeness of this method of working. None the less, it served the purpose of showing that carbitol thiocyanate has a marked fumigant effect on some insects. This method also gave high kills with such well-known fumigants as formalin and carbon disulphide and low kills with the vapour from pyrethrum insecticides (cf. Ginsburg, 1930). Mercury vapour had very little effect.

The conclusion arrived at from these experiments therefore is that β-butoxy-β'-thiocyanodiethylether is toxic to some insects in its vapour phase.

(c) *The effect of films in oil*

In earlier work (Potter, 1938) on the control of insect pests of stored goods it was found that one of the most important properties of a spray was its residual effect as a film. The importance of this residual effect had not previously been realized. The properties of insecticide films of pyrethrum in white oil have been investigated to some extent (Potter, 1938) and it was thought that preliminary experiments with the thiocyanate on similar lines would be useful.

The chief factors influencing the residual effect of an insecticide as a film appear to be

(1) The concentration of the toxic material.
(2) The nature of the carrier of the toxic material.
(3) The amount of the insecticide forming the film.
(4) The nature of the surface to which the film is applied.
(5) The age of the film.
(6) The period of exposure of the insect to the film.

Of these factors (2) and (4) were kept constant. The carrier of the thiocyanate was Shell oil 24210, the specification of which has already been given. The films were applied to 4 in. squares of wood, Canadian hemlock in all instances except one, where a deal of unknown origin was used. The experiments detailed below were then made in order to obtain some indication of a concentration of thiocyanate, which would be effective after a reasonable space of time with a film which could be formed in practice. The effect of the film was tested on a number of species of insects and some knowledge was gained of its lasting properties.

Experimental

The insecticide consisted of the thiocyanate diluted with Shell oil 24210 to give the desired concentration of β-butoxy-β′-thiocyanodiethylether. The heavy oil was used because this was thought to give the best effect as a film, owing to its high viscosity and low volatility.

Squares of wood (4 in.) were cut out, smoothed with emery paper and weighed. They were then placed in the spraying tower, sprayed and afterwards weighed again to find the amount of spray deposited. After a little practice films of a known weight could be repeated with only a small variation. A 3 in. circle was drawn on each square, in which the insects were placed and then covered with an inverted filter funnel. The open end of the filter funnel was covered with muslin and the periphery of the funnel was cemented to the wood with plasticine. With this arrangement the insects could not escape and a lethal concentration of vapour would not be built up. After the insects had been exposed for the requisite time they were removed to tubes and kept either in the laboratory or in an incubator at a constant temperature and humidity.

The insects used were not standardized in any way; they were bred under a variety of conditions and their ages were not known.

Table 6 shows the effect of films on a variety of insects that attack stored products. Adults of *Oryzaephilus surinamensis* L., and *Lasioderma serricorne* F., proved very susceptible, both species being badly affected after an exposure of 5 min. Moths of *Ephestia kühniella* Z., and *Tineola biselliella* Hum., do not appear to be very susceptible, because, although a kill of 100% or near it was obtained, the exposure necessary before all the specimens were affected

was considerable. Comparison with previous work (Potter, 1938) suggests that thiocyanate is not as effective as pyrethrum in this respect.

It appears possible, using a concentration of 3 % thiocyanate with fairly heavy fresh films of 0·020 g./sq. in. and an exposure of 24 hr., to obtain a high percentage kill of a number of the more important insects attacking stored products.

In view of the importance of the grain weevils *Sitophilus granarius* L. and *Sitophilus orzae* L., a few experiments were made on the effect of films of the thiocyanate on the adults of these two species. The results are shown in Table 7. A 5 % solution appears to be necessary to secure a high percentage kill for exposures up to 24 hr. It is possible that with heavier

TABLE 6. *Effect of films of the thiocyanate in Shell oil 24210 on deal on various insects*

Species of insect used	No. used	Thiocyanate %	Amount of insecticide g./sq. in.	Age of films (hr.)	Period of exposure to film (hr.)	% kill
Caulophilus latinasus Say.	51	3	0·020	New	24	93·7
adult	50	Control	—	—	—	14
Tribolium castaneum Hbst.	52	3	0·020	New	24	100
adult	50	Control	—	—	—	2
Lasioderma serricorne F.	53	3	0·020	New	24	100
adult	50	Control	—	—	—	2
Oryzaephilus surinamensis L.	46	3	0·020	New	24	100
adult	50	Control	—	—	—	0
Tineola biselliella Hum.	103	3	0·010	New	½	98·1
adult						
Ephestia kühniella Z. adult	33	3	0·010	New	24	100
	11	Control	—	—	—	0
	7	5	0·017	24	1	100
	11	Control	—	—	—	0
Rhizopertha dominica Dom.	25	12	0·007	24	1½	36
adult	25	Control	—	—	—	0
Plodia interpunctella Hb.	24	12	0·007	New	24	91·7
larvae	25	Control	—	—	—	16

films the percentage of thiocyanate might be reduced. The lasting properties of the films appear to be quite good, because they are toxic after 120 hr. It is desirable that further experiments should be made on heavier films of low dilutions of thiocyanate.

A final series of experiments was made on the effect of films on the bed-bug, *Cimex lectularius* L., and the results are shown in Tables 8–12.

Table 8 shows the effect of light films, containing a high percentage of thiocyanate, on the adults. A high percentage kill can be obtained with short exposures and the films retain a high degree of toxicity after 9 days. Treated insects were able to lay fertile eggs before they died.

Table 9 shows the result of an experiment with a 12 % thiocyanate film on the eggs of *Cimex*. The eggs were left on the film until they either hatched or died. In this instance all the eggs were killed. It is noteworthy that nymphs formed inside the chorion of the eggs but none was able to hatch.

These two series of experiments were only preliminary, since 12 % is too high a concentration for practical use. Table 10 gives the results of a series of experiments on the effect of films of 6 % thiocyanate on the adult *Cimex lectularius* L. and shows that 100 % kill may be

obtained with this concentration with new films varying from 0·006 to 0·013 g./sq. in. The toxicity of the light film, however, falls off very rapidly, while that of the heavier films is retained very much better. These experiments indicated that the films that had been used

TABLE 7. *Effect of films of the thiocyanate in Shell oil* 24210 *on deal on adult* Sitophilus granarius *L. and* Sitophilus Oryzae *L.*

Species of insect used	No. used	Thiocyanate %	Amount of insecticide g./sq. in.	Age of film (hr.)	Period of exposure to film (hr.)	% kill
Sitophilus granarius L.	46	3	0·020	New	24	61·7
	50	Control	—	—	—	0
Sitophilus oryzae L.	53	3	0·010	New	24	45·3
	50	Control	—	—	—	0
Sitophilus granarius L.	25	5	0·019	24	24	100
	28	5	0·023	24	24	100
	25	Control	—	—	—	11·5
Sitophilus oryzae L.	27	5	0·019	24	24	74·1
	24	5	0·023	24	24	70
	27	Control	—	—	—	14·8
Sitophilus granarius L.	31	6	0·006	24	24	90·3
	25	6	0·009	24	24	100
	25	6	0·013	24	24	100
	21	Control	—	—	—	19
	25	6	0·006	120	24	16
	25	6	0·009	120	24	52
	25	6	0·013	120	24	64
	26	Control	—	—	—	7·7
	100	12	0·006	New	15	95
	100	Control	—	—	—	6

TABLE 8. *Effect of films of* 12% *thiocyanate in Shell oil* 24210 *on adult* Cimex lectularus *L. Films applied to* 4 *in. squares of Canadian hemlock*

No. used	Amount of insecticide g./sq. in.	Age of film (hr.)	Period of exposure to film (hr.)	% kill
20	0·0070	New	2	85
25	Control	—	—	24
24	0·0070	New	15	87·5
25	Control	—	—	15
25	0·0070	24	15	96
25	Control	—	—	16
25	0·0065	216	14	96
25	Control	—	—	0

TABLE 9. *Effect of films of* 12% *thiocyanate in white oil on eggs of* Cimex lectularius *L. Films applied to* 4 *in. squares of Canadian hemlock*

No. used	Amount of insecticide g./sq. in.	Age of film (hr.)	Period of exposure to film (hr.)	% kill
25	0·0070	192	Left on until dead	100
25	Control	—	—	0

previously were too light and a further series was made with 5% thiocyanate to compare the action of films of different weights.

In the first series (Table 11) it appears that films of 0·019–0·023 g./sq. in. are highly toxic after an interval of 120 hr.

Table 12 shows the effect of similar films on nymphs in various stages of development. Some of these stages are more resistant to the films than adults, because there is a fair percentage recovery from the 120 hr. old films.

The series of experiments on the 5 % thiocyanate were considerably more extensive than is

TABLE 10. *Effect of films of* 6 % *thiocyanate in Shell oil* 24210 *on adult* Cimex lectularius *L. Films applied to* 4 *in. squares of Canadian hemlock*

No. used	Amount of insecticide g./sq. in.	Age of film (hr.)	Period of exposure to film (hr.)	% kill
27	0·006	New	24	100
25	Control	—	—	0
26	0·009	New	24	100
25	Control	—	—	8
25	0·013	New	24	100
25	Control	—	—	8
25	0·006	48	24	48
26	0·009	48	24	100
29	0·013	48	24	93·1
37	Control	—	—	5·4
27	0·006	120	24	14·8
26	0·009	120	24	73·1
25	0·013	120	24	72
25	Control	—	—	0
26	0·009	168	24	65·4
21	0·013	168	24	76·2
25	Control	—	—	8

TABLE 11. *Effect of films of* 5 % *thiocyanate in Shell oil* 24210 *on adult* Cimex lectularius *L. Films applied to* 4 *in. squares of Canadian hemlock*

No. used	Amount of insecticide g./sq. in.	Age of film (hr.)	Period of exposure to film (hr.)	% kill
19	0·016	24	24	94·7
25	0·019	24	24	100
27	0·023	24	24	100
25	Control	—	—	0
26	0·019	48	24	96·14
27	0·023	48	24	100
25	Control	—	—	0
30	0·019	120	24	100
28	0·023	120	24	96·4
25	Control	—	—	4

shown in Tables 11 and 12, but it was found that the stocks of *Cimex lectularius* L. were losing resistance and to quote the further results would be misleading. No reason could be found for the loss of resistance.

These preliminary series of experiments on bed-bugs indicate that β-butoxy-β'-thiocyanodiethylether is a promising insecticide for use against these pests, because the films of insecticide formed would be toxic to all stages of the insect, including the egg.

Fertile eggs were laid by a number of the insects that were killed by the film, but the eggs in their turn are likely to be killed if they are laid and left on surfaces covered with the film.

TABLE 12. *Effect of films of 5 % thiocyanate in white oil on nymphs of* Cimex lectularius *L. (various ages). Films applied to 4 in. squares of Canadian hemlock*

No. used	Amount of insecticide g./sq. in.	Age of film (hr.)	Period of exposure to film (hr.)	% kill
31	0·019	48	24	100
32	0·023	48	24	100
25	Control	—	—	0
29	0·019	120	24	55·2
24	0·023	120	24	75
25	Control	—	—	4

DISCUSSION

The experiments described in this paper indicate that β-butoxy-β′-thiocyanodiethylether has distinct possibilities as an industrial insecticide. We have, however, no information about optimum dosage. This is important because it appears to be possible to obtain similar kills either by means of a high concentration and a low dosage or by a low concentration and a high dosage.

Much of the work was done with the bed-bug, *Cimex lectularius* L., which was found to be susceptible to the insecticide whether applied in the form of a spray, a vapour or a film. The adult and nymphal bugs were very susceptible to the oil diluent alone, as a spray. A film of 6 % thiocyanate killed 100 % of nymphs and adults. The eggs were particularly susceptible: ½ % thiocyanate gave a very high kill with a small dosage and this susceptibility of the eggs has been confirmed in later work. The eggs were also found to be susceptible to a high degree to a very light film containing 12 % thiocyanate.

Adults of the grain weevil, *Sitophilus granarius* L., and the rice weevil *S. orzae* L., appeared to be effected by 3 % thiocyanate as a spray and results suggest that 5 % thiocyanate in a heavy oil as a fresh film should give high kills.

As a film the insecticide appeared to be toxic to *Caulophilus latinasus* Say., *Lasioderma serricorne* F., *Oryzaephilus surinamensis* L., and *Tribolium castaneum* Hbst.; *Lasioderma serricorne* F., and *Oryzaephilus surinamensis* L., appeared to be particularly susceptible.

The results of the experiments on films of thiocyanates may be compared with the results of experiments on pyrethrum films performed under similar conditions (Potter, 1938).

Such a comparison must be based on the concentrations of each insecticide that it is economical to use in practice, and on this basis the thiocyanate films appear to have a similar order of toxicity to pyrethrum films to the bed-bug, the grain and rice weevils and the other species of beetles attacking stored products that were tested; and owing to the superiority of the thiocyanate as a fumigant it may prove more effective against these insects in the field. The thiocyanate films appear to act more slowly than the pyrethrum films and on this account they do not appear to be as effective against the adult lepidoptera that were tested. Insufficient data are available to form a judgement on their comparative action on larval lepidoptera.

One important result of our investigations has been to show the marked fumigation effect of carbitol thiocyanate and to produce evidence of its superiority in this respect over both pyrethrum and dodecyl rhodanate. This property should be important as it may enable

the insecticide to kill insects which cannot be reached by ordinary spraying. The eggs of the bed-bug seemed to be especially susceptible to the fumigation effect.

The question of the health hazard of this insecticide has been investigated by Cameron *et. al.* (1939).

Summary

Preliminary experiments to investigate the toxicity of β-butoxy-β′-thiocyanodiethylether (normal butylcarbitolthiocyanate) to some industrial and household insect pests are described. The insecticide was diluted with a light and a heavy oil, specifications of which are given.

The insecticide was found to be toxic as a spray and had a marked effect in its vapour phase (except on *Tenebrio molitor* L.); under suitable conditions it could also be made to form a toxic film on wooden surfaces.

The thiocyanate appeared to be particularly toxic to the eggs of the bed-bug, *Cimex lectularius* L.

The following were used as test insects: *Sitophilus granarius* L., *S. oryzae* L., *Caulophilus latinasus* Say., *Lasioderma serricorne* F., *Oryzaephilus surinamensis* L., *Tribolium castaneum* Hbst., *Plodia interpunctella* Hb., *Ephestia kühniella* Z., *Cimex lectularius* L., *Tenebrio molitor* L., *Dermestes vulpinus* F., and *Ephestia elutella* Hb.

Of these insects all, except *Dermestes vulpinus* F. and *Tenebrio molitor* L., which were used only in experiments on the toxicity of the vapour, were susceptible in varying degrees to the thiocyanate in one form or another.

The thiocyanate appears therefore to have considerable promise as an industrial insecticide either alone or mixed with other toxic substances, e.g. pyrethrum or derris.

REFERENCES

Badertscher, A. E. (1936). Insecticide tests compared. *Soap* (9), **12**, 96, 97, 101.

Bousquet, E. W., Salzberg, P. L. & Dietz, H. F. (1935). New contact insecticides from fatty alcohols. *Industr. Engng Chem.* **27**, 1342.

Cameron, G. R., Doniger, C. R. & McKenny Hughes, A. W. (1939). The toxicity of lauryl thiocyanate and *n′*-butylcarbitol-thiocyanate (lethane 384). *J. Path. Bact.* **49**, 363.

Ginsburg, J. M. (1930). Effect of pyrethrum vapours on insects. *Rep. N.J. agric. Exp. Sta.* 1928–9, p. 176.

Kearns, H. G. H. & Martin, H. (1935). Investigations on egg killing washes. The ovicidal properties of lauryl rhodanate. *Rep. agric. hort. Res. Sta. Bristol*, p. 49.

Musgrave, A. J. & Mackinnon, D. L. (1938). Infection of *Plodia interpunctella* Hb. with a Schizogregarine, *Mattesia dispora*. *Proc. R. ent. Soc. Lond.* A, **13**, 89.

Potter, C. (1938). The use of protective films of insecticide in the control of indoor pests with special reference to *Plodia interpunctella* Hb. and *Ephestia elutella* Hb. *Ann. appl. Biol.* **25**, 836.

Tischler, N. & Stonis, J. (1938). Synthetic insecticides. A further study of the value of naphthyl isothiocyanate in fly sprays. *Soap* (10), **14**, 97, 99.

Tischler, N. & Viehoever, A. (1938). A new synthetic insecticide. *Soap* (10), **14**, 117, 119, 121, 123.

Wilcoxon, F. & Hartzell, A. (1935). Further experiments on the organic thiocyanates as insecticides. *Contr. Boyce Thompson Inst.* **7**, 29.

Woodbury, E. N. (1938). Tests methods on roaches. German cockroach as a test insect for liquid petroleum insecticides. *Soap* (8), **14**, 86–90, 107, 109.

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MATERIALS FOR A STUDY IN ANIMAL COMPETITION. THE FAUNA OF THE SEWAGE BACTERIA BEDS

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(With 4 Text-figures)

Introduction

The bacteria beds of sewage works would rapidly become choked and useless but for scouring organisms that have established themselves in these recently created environments. Few species have successfully colonized the beds, and these prosper there exceedingly. They comprise six species of Diptera, two of Collembola and two oligochaete worms. There is evidence of an active competition amongst them (Lloyd, 1935, 1937) providing many problems for study and analysis, and information has now been collected that makes it possible to deal with some of these. There has been systematic and almost uninterrupted trapping of the emerging flies for more than five years coupled with a laboratory study of their fecundity and rate of progress at various temperatures. In this way an idea of what the seasonal incidence of the various species should be has been obtained, and deviations can be attributed to the pressure of the competing forms where no other evident factor is to be found.

Two aspects of this competition are dealt with particularly. First, do the various forms reduce one another by predacity? None of the species dealt with is a definitive predator, but in a time of scarcity of normal food many insects will change their habit in this regard. Secondly, to what extent are the larvae, pupae, and worms with their cocoons washed out of the beds? Is this loss regular or seasonal, and does it affect some species more than others? This factor is of some economic importance, since the scouring organisms are wholly beneficial in the beds but, when they are drowned in the final settlement tanks, their putrefying bodies cause deterioration of the purified effluent as shown by Welch (1914) for enchytraeid worms. A routine straining has been carried out through one year to obtain information on this point.

These observations have been made mainly at the Knostrop Sewage Works of the Leeds Corporation with the active collaboration of the Manager, Mr J. T. Thompson.

Material and methods

The Knostrop works

The beds are rectangular, 6 ft. in depth, and the medium is 1–3 in. water-worn gravel of an open nature at the surface but more compacted in the depths. They are floored with convex tiles which cover gently sloping runlets, and these discharge into a concealed channel running the length of the bed. They are in terraces 150 yd. long and 40 yd. wide, and the terrace is sprayed by four machines whose traverses almost meet in the centre leaving very narrow strips of medium unsprayed at the ends of the terraces, the whole length between the spraying arms, and across the middle where the machines reverse their traverse. Along the length they are bounded by raised channels carrying the sewage supply of adjoining terraces and the rails on which the machines run. At the ends the terraces have low walls beyond which are paths and grassed banks. Certain of these features are of importance in understanding the distribution of the flies.

The sewage

Most of the solids in suspension in the crude sewage are removed by screens and sedimentation in tanks. The water from the sedimentation tanks is delivered on to the bed in a sheet by the distributors. The water head is about 5 ft., so the water strikes the pebbles with considerable force but at once becomes a mere trickle and the percolation is very gentle, a mere film over the medium. When storm water allows of it, the beds are rested for some hours in the night and rests of 3 days' duration are given occasionally, but only the top few inches of the medium dry in these longer rests, dependent on weather conditions. The purified sewage collects into gullies at the ends of the terraces, and flows in these with much force to the final settlement tanks where flow is slow and the humus (solids derived in passage through the bed) settles down before the final effluent passes to the river.

Solids in the effluent

The solids in the tank effluent do not vary greatly from day to day or season to season. The solids in the bed effluent do vary greatly, and this variation is due to fluctuating activity of the scouring organisms. This variation at Knostrop has the range of 3–19 parts per 100,000 by weight. The record for the year of the straining experiment is included in Table 1, as it has an important bearing on the observations.

Bed growth

This is of two main types. First, there is a leathery growth of *Phormidium* (Cyanophyceae) on the surface with a slight admixture of *Ulothrix* (Chlorophyceae) and other algae. A study of this growth has been made by Reynoldson (1939) who took weekly scrapings of 1 sq. ft. of surface and recorded the dry weight (Table 1). The *Phormidium* is subject to thickenings which rupture it, and to peeling and fragmentation by sun and wind during bed drying, and to loosening by occasional frost. Fragments of it, therefore, break off and are washed down into the bed where they are eaten by larvae and worms. It is also subject to attack *in situ* by the enchytraeid worms which invade it in masses and pulverize it till it may be entirely destroyed. This occurs especially in spring when the worms make a great invasion of the growth already somewhat loosened by the events of winter. Thereafter, the growth reforms and normally more than keeps pace with the destructive forces through the summer. The second type of growth is in the depths and is a slimy zooglaea of the purifying bacteria, fungi, Protozoa and other organisms. These have been listed on several occasions (Johnson, 1914; Lackey, 1924, 1925). On this growth, as well as on the washed-down fragmented alga and the finely divided solids of the tank effluent, the larvae and the worms in the depths subsist.

Bed fauna

The larger members of the fauna common in the beds observed are as follows, an asterisk marking those subjected to special study:

OLIGOCHAETA

Lumbricidae: *Lumbricus rubellus* Hoff.
Enchytraeidae: **Lumbricillus lineatus* Mull.
Naididae: *Nais sp.* in channels below the beds.

MOLLUSCA

The field slug, *Agriolimax agrestis* L., is moderately abundant at the surface, but no other species of slug has been seen there. *Limnaea glabra* Mull. abounds.

INSECTA

Diptera

Psychodidae: **Psychoda severini* Tonn., **P. alternata* Say.
Chironomidae: **Metriocnemus longitarsus* Goet., **M. hirticollis* Staeg., **Spaniotoma* (*Limnophyes*) *minima* Mg., **S.* (*Orthocladius*) *perennis* Mg. The two following breed freely in the channels but not in the beds, both having tube-making larvae: *Chironomus dorsalis* Mg., *Tanytarsus atrofasciatus* Kieff.
Cordyluridae: **Spathiophora hydromyzina* Fln.

Collembola

Achorutes viaticus Tulb. and *Tomocerus minor* Lubb., very common scourers at some works, are rarely encountered at Knostrop.

Arachnida

A gamasid mite is sometimes abundant but has not yet been studied and its status in the fauna is not known.

Eighteen species of spiders all of the Linyphiidae have been taken and are the only definitive predators of importance in the beds. Of these several species of *Lessertia*, *Porrhomma* and *Leptyphantes* are rather abundant and Dr W. S. Bristowe (1939), who kindly surveyed the series, says that they have hitherto been regarded mainly as cellar forms.

Offloading

This phenomenon is most pronounced in spring and is characterized by the destruction of the growths described above with a consequent increase in the solids of the bed effluent which temporarily deteriorates. It is due to the onset of spring activity by the scourers. It is, of course, really a continuous process, but at this season destruction more than balances regeneration. The process is beneficial, and at some works is artificially induced at other seasons by the judicious use of bleaching powder. Apart from the radical purging of the medium it no doubt keeps the nitrifying bacteria "physiologically young" as the ciliate *Colpidium* is supposed to do in cultures (Meiklejohn, 1932). During the year under survey the spring offloading was rather earlier than usual and by no means complete. When the process is radical the surface becomes quite clean and there follows a phase when the beds assume the appearance of distant lawns owing to the growth of *Ulothrix* which precedes the more characteristic development of *Phormidium*. A result of the offloading is a temporary shortage of food for the scourers, at that time actively reproducing.

Bed temperature

Mr Thompson has recorded temperatures over long periods in connexion with this work. The temperature of the water fed to the beds (monthly means, 9·0–18° C.) and in the effluent gullies is almost identical through the year. It follows that in cold weather the beds are warmed by vital activities. The bed temperatures are uniform from 6 in. downwards. In midwinter they are warmer than the mean shade temperatures by some 5° C. In very hot weather they are cooler than the mean shade by 1–2° C. A more detailed account of the bed temperatures has been given by Lloyd (1937). Those for the period covered by this paper are given in Table 1.

Estimate of fly output

This is estimated by trapping the emerging flies in shallow wood trays of 1 sq. ft. area pierced by a central hole over which rests a glass jar with a perforated inverted paper cone. The jars include corrugated card to afford the insects resting places. The jars are changed twice weekly and the trays moved to fresh sites once weekly. Three traps are in use, and the average catches of the three during the straining experiment are given in Tables 2 and 3 under the specific headings. In the text, unless otherwise stated, all fly estimates are means from the three traps. The "rough estimate" given in addition for *Psychoda alternata* is of a different type for reasons given below.

Estimate and distribution of worms and larvae

For *Lumbricillus lineatus* counts have been made twice weekly in 20 g. of the growth scraped from the surface. Fly larvae are scarce in this. The distribution in the depths has been found by placing muslin bags containing 20 g. of scalded *Phormidium* in the bottoms of lidded pipes sunk to a depth of 1 ft. and 2 ft. 6 in. respectively. The bags were removed once weekly and the worms and larvae counted. The record is summarized in Tables 1–3. More detail is given by Reynoldson (1939).

Bed effluent straining

In order to observe the extent to which the scouring organisms pass out of the beds, a straining was carried out in the effluent gully at weekly intervals throughout a year. It has been mentioned that the force of water in these gullies is considerable, and it is impracticable to do more than sample it without any idea of the volume really strained. The conditions of straining were uniform and the results are strictly comparable one with another. The strainer, which was placed against a sluice gate, consisted of a board with a round opening 6 in. in diameter in which the net fitted. This net was 18 in. long, composed of a perforated zinc cylinder fitting into the board and covered with sacking. To this was attached

a bag of "double extra heavy" bolting silk, mesh 84/1 in. with a collecting jar in the mouth. Water was able to pass below the board in the gully, and the backwash from the net was considerable. The large lumbricid worms passed below the board, but the jar retained a satisfactory sample of the solids in the effluent with the organisms required. The standard time of straining was 5 min. The concentrate was taken to the laboratory and placed in a large flask which was then filled with a solution of magnesium sulphate, 320 g./l. This brings the larger organisms to the surface and is the method developed by Ladell (1936) for separating insects from soil. The insect larvae and pupae, the worms and their cocoons, were then sorted and counted. They were grouped as shown in Tables 1–3, the pupae of *Psychoda* spp. being counted together. Dead and fragmentary material was ignored and young larvae would be missed. In all, fifty such strainings were made, and in the tables the first record is given separately as representing a transition period and the last, for which there was no record of "solids", while the others are averaged to four-weekly periods. All the other data in the tables are made to conform to the same periods except that the records of fly output are arranged to begin just after the date of straining, e.g. record of strainings on 29 April, 6, 13 and 20 May are averaged, and the fly output in the corresponding column is for a weekly average from the three traps from 30 April to 27 May and so on. The deficiencies of this method are recognized, but it has answered quite well in practice and it is the best that could be arrived at without attempting to age the larvae strained and employing an elaborate sliding scale for their emergence as flies. The pupal periods are all very short, a few days only, and inspection of the durations of the total life cycles given later in relation to the bed temperatures will show that for most of the year a larva of any of the species would reach emergence from half-growth in under a month. The majority of the larvae counted were older than this, and the emergence of most of those of corresponding age remaining in the beds should be covered by the approximations adopted.

Correlations

The groups of data collected in Tables 1–3 have been studied by working out the coefficients of correlation wherever it appeared that one factor might bear upon another, the material being treated as "unsorted data" (Woods & Russell, 1931). Some degree of correlation is indicated when the coefficient is between 0·5 and 1·0, the sign + or −, and the nearer to unity the more close the correlation. These coefficients are gathered together in Table 6 and are referred to below in this manner: "They give a correlation with the solids in the effluent (+0·755)."

Life cycles and culture

Much information about the organisms has been gained by culture at graded temperatures well beyond the range of any that the insects can encounter in the beds. Cultures are kept in jars on pads of waterlogged cotton-wool from which foul water can be withdrawn by a pipette. The food is the surface growth of the bed, scalded and kept aerated till its tendency to putrefy is reduced. For most of the stages of the more abundant insects the thresholds of development and the thermal constants have been given (Lloyd, 1937), and as there is no diapause in the cycles, an inclusive threshold and constant can be framed for the average whole life cycle which answers well for what are middle temperatures for the species. The whole life cycle must be understood to include maturation of the female, oviposition, incubation, larval growth and pupation. Inclusive average times for these are given in the account below, the temperatures being means with mostly a range of 2–3° C. At low temperatures within the possible range of development of a phase the speed is, in many cases, greater than the formula demands (Uvarov, p. 26, 1931). In our experience with these sewage insects this acceleration just above the theoretical threshold has been invariable, but the insect in achieving speed usually suffers undue mortality (Lloyd, 1937). This mortality is reflected in the average family size at various temperatures given below, the family being the total offspring in culture of the single female fly. It is not suggested that in nature, food being adequate and interference nil, these family sizes would be followed, but it seems reasonable to suppose that if an insect encounters a difficulty at a certain temperature in culture, some adverse effect at least from the same degree of temperature would be felt under otherwise normal conditions.

Predacity tests

Direct observations on predacity are difficult in the beds because all the larvae shun the light and are disturbed when exposed. There is no doubt that food gets very scarce in the beds at certain times, and it was desired to know whether the insects would attack one another under these conditions and whether some species were more apt to do this than others. These questions have been answered by

laboratory experiment. The species have been taken seriatim. One or two hungry larvae, generally third instar, were placed in a glass capsule with a wisp of wet cotton-wool and given access to either the eggs, larvae or pupae of their own or of a competing species till nearly all combinations had been covered. *Lumbricillus lineatus* and its cocoons also were made potential prey. The tests were prepared as follows: (1) *Eggs: Psychoda* spp. and *Spathiophora hydromyzina* were induced to oviposit a full batch in the wisp of wool while the eggs of the chironomids in their undamaged mucous envelopes were placed on the wool surface. The egg cocoons of *Lumbricillus lineatus* were taken from culture and laid on the wool. (2) *Larvae*: These or the worms were added from stock cultures. (3) *Pupae*: The *Psychoda* were allowed to pupate in the wool or were added from culture, while the larvae of the chironomids were allowed to complete development in the capsules so that their cocoons should be unbroken and any excess food was removed before the rival larvae were added. The experiments were carried out at room temperature, generally about 15–17° C., until either the eggs hatched, larvae died or pupated, and pupae became flies. The experiments gave differential results which are analysed below under the species used. The ratio of the number of tests showing definite predacity, or damage only, to the total number of tests made is used as an index, e.g. 5 cases of attack in 12 tests = 5/12.

General account

In the following account the organisms studied are dealt with, and under each are given first notes on their general behaviour in nature, their temperature relationships, speed of progress and family size where known from cultures, their distribution in the beds according to the known concentration, and their seasonal incidence. Then follows their incidence in the strainings with the correlations and finally the observations on predacity.

Lumbricillus lineatus

An account of the behaviour of this worm is given by Reynoldson (1939, 1939*b*). At Knostrop it is the most important of the scourers as it controls the surface growth that would otherwise choke the beds. Its chief breeding season is in the cooler months, but the total population does not vary conspicuously during the year although the position of concentration varies. The worms recede from the surface for a few inches in cold weather, and when they have reduced or destroyed the *Phormidium* in spring they go much deeper, following their food. These movements are shown in Table 1, and inspection of the seasonal figures shows that the deep descent of the aggregations, below 1 ft., occurs not at low but at middle temperatures. The data have been examined by the coefficients of correlation (see p. 125) between temperature, surface growth and concentration of the worms with the following results: the worms in the surface growth and the concentrations at 1 ft. give correlation with temperature (+0·670 and +0·696 respectively), those at the 2 ft. 6 in. depth do not (+0·487); those at the surface do not correlate with the development of surface growth (+0·452), but those in the depths give negative coefficients, at 1 ft. (−0·628) and at 2 ft. 6 in. (−0·757). Thus they enter the *Phormidium* when conditions are warm enough, but they descend deeply when the surface *Phormidium* becomes scanty. In very cold weather they wait just below the surface ready to assail the alga whenever a warm spell allows it.

The cocoons are deposited in the *Phormidium* or are attached firmly to the pebbles, and it is considered that this property, not attributed to any other *Lumbricillus*, may be one of the selective characters that allow this worm to colonize the beds. The worms appear in the strainings in considerable numbers which correlate with the amount of solids (+0·626) and with the indications of worm concentrations at 1 ft. (+0·795) and at 2 ft. 6 in. (+0·684). There is negative correlation with the amount of *Phormidium* (−0·606), but none with the

seasonal abundance of the worms in this (−0·007). Relative to the worms the cocoons do not appear in the strainings in large numbers, but these show much the same coefficients of correlation as the worms themselves (see Table 6). These figures seem to explain the seasonal discharge of the scouring organisms from the beds. Spring activity causes a shortage of food in the upper layers, and the organisms go deeper in search of sustenance till they get into a position where they pass through the bed bottom, or are discharged by the escaping water.

TABLE 1. *Record of worms and cocoons,* Lumbricillus lineatus, *counted in strainings of the bed effluent with corresponding indications of worm abundance in the beds, mean bed temperatures, solids in the effluent and dry weight of surface growth. The first item and the last cover single weeks, the other items* 4 *weeks, averages*

Dates of strainings	Mean bed temp. ° C. at 3 ft. 0 in.	Dry weight surface growth g./sq. ft.	Solids in effluent per 100,000 by weight	*Lumbricillus lineatus*				
				Worms at surface	Worms at 1 ft. deep	Worms at 2 ft. 6 in. deep	Worms strained	Cocoons strained
1937 22 Apr.	11·6	3·39	6·6	2570	958	1699	—	—
29 Apr., 6, 14, 20 May	12·5	2·54	5·0	825	743	3750	72	7
27 May, 3, 10, 17 June	16·6	1·67	9·3	1310	4030	3263	272	15
24 June, 1, 8, 15 July	16·6	2·26	9·9	1082	2316	1833	90	7·5
23, 29 July, 5, 12 Aug.	16·1	3·76	5·3	4767	2875	1748	112	4·0
19, 26 Aug., 9 Sept.	17·1	6·34	4·5	6343	1217	764	26	0·7
16, 23, 30 Sept., 7 Oct.	15·1	6·83	3·2	5423	485	349	43	1·5
14, 21, 28 Oct., 4 Nov.	13·0	7·08	5·7	3556	166	92	7·7	0
11, 18, 25 Nov., 2 Dec.	9·8	5·82	4·9	859	36	146	6·7	0·5
9, 16, 30 Dec.	7·6	5·52	3·1	391	22	115	4·7	0·7
1938 6, 13, 20, 27 Jan.	8·6	5·10	6·1	·514	77	157	2·5	1·5
3, 10, 17, 24 Feb.	7·5	3·94	3·5	567	160	194	58	3·2
3, 10, 17, 24 Mar.	11·1	3·40	3·5	702	570	379	43	1·7
31 Mar., 7, 14, 21 Apr.	11·1	5·59	5·7	833	1050	817	93	2·0
28 Apr.	14·6	5·42	—	3300	400	962	153	1
Totals strained out							3471	178

Psychoda severini

This fly is parthenogenetic in the beds and we have never seen the male though it is known (Tonnoir, 1922; del Rosario, 1936). It lays some 90–120 eggs inserted singly or in small clusters into the food. The time of its life cycle, except at very low temperatures, agrees with a threshold of 1·7° C. and a thermal constant of 480° C. Fully favourable temperatures are from 8 to 18° C., and the optimum is about 10–15° C. The following is representative of laboratory experience of timed life cycles and family size:

2·5° C. 200 days: 3 families, 12 ± 5·5, 0 failed.
1·5–3·0° C. for 130 days then 14° C.: 12 families, 13·4 ± 4·2, 0 failed.
7° C. 90 days: 3 families, 37 ± 5·2, 0 failed.
10° C. 60 days: 15 families, 68 ± 8·5, 1 failed.
15° C. 36 days: 30 families, 52 ± 4·9, 1 failed.
20° C. 26 days: 15 families, 35 ± 7·8, 0 failed.
24° C. 22 days: 24 families, 16 ± 2·2, 0 failed.
25·5° C. 14 attempts, 13 failed, 1 family of 14 puny and crippled, the mortality is mainly larval.
26° C. 21 attempts, all larvae died.

At Knostrop the adult is abundant only from March to June or July, though the bed temperatures are normally within the favourable zone during nearly the whole year. It reaches its maximum in April or May and then declines rapidly, and in some summer months has not been seen at all. This contrasts strongly with the conditions at Barnsley, where its maximum flight is from March to May, but it remains abundant throughout the year (Lloyd, 1937). This continued abundance gives it a better start in the winter months when *Psychoda alternata* and *Spaniotoma minima* (usually) are depressed.

TABLE 2. *Record of larvae and pupae of* Psychoda alternata, P. severini *and* Spaniotoma perennis *counted in strainings of the bed effluent with corresponding indications of abundance, flies emerging on* 1 *sq. ft., larvae trapped in the depths. The first item and the last cover single weeks, the other items* 4 *weeks, averages*

	P. alternata					*P. severini*		*S. perennis*		
Dates of strainings	Fly on surface Tray estimate	Larvae at 1 ft. deep	Larvae at 2 ft. 6 in. deep	Larvae strained	Pupae strained mainly *P. alternata*	Fly on surface	Larvae strained	Fly on surface	Larvae strained	Pupae strained
1937 22 Apr.	130	—	—	39	2	55	34	1·3	0	0
29 Apr., 6, 14, 20 May	260	—	—	122	12	118	44	3·0	4·2	2·2
27 May, 3, 10, 17 June	200	0	13	152	16	40	38	1·4	9·2	0·2
24 June, 1, 8, 15 July	140	5·3	25	72	6·2	5·6	3·5	0	0·5	0·2
23, 29 July, 5, 12 Aug.	90	1·0	167	34	9·7	4·1	0	0	1·0	5·2
19, 26 Aug., 9 Sept.	320	36	79	6·3	0·7	0·1	0	0	0	0
16, 23, 30 Sept., 7 Oct.	250	9·0	77	24	5·5	0·1	0	0	0	0
14, 21, 28 Oct., 4 Nov.	420	3·7	2·5	8·5	0·5	0·5	0	0·2	0	0
11, 18, 25 Nov., 2 Dec.	60	2·5	5·5	2·5	0·5	0·2	1·2	0·2	0	0
9, 16, 30 Dec.	65	0	0·2	5·0	1·7	2·2	2·7	0·7	0	0
1938 6, 13, 20, 27 Jan.	200	0·2	5·5	12·2	1·2	0·8	2·5	0	0	0
3, 10, 17, 24 Feb.	490	0·5	3·5	98	12·7	3·5	9·0	0	0·2	0
3, 10, 17, 24 Mar.	900	8·7	162	164	27	25	28	0	0	0
31 Mar., 7, 14, 21 Apr.	3200	50	243	212	69	45	20	1·0	0	0
28 Apr.	1300	3	64	106	81	60	48	1	0	0
Totals strained out				3782	734		675		61	32

The larva is sluggish, adapted for immersion in semi-fluid food with the syphon projecting, and the pupa is adapted for the same location, but in the beds both often lie free or in crevices. Neither the larva nor the pupa, which moves about when disturbed, have much prehensile power. The pupae particularly may be dislodged by the trickle of water, and in their time of abundance both stages pass out of the beds in great numbers (Table 2). Their zone of concentration has not been located at Knostrop, but at Huddersfield it is found to be

near the surface (p. 144). The same tendency to concentration is doubtless present at Knostrop, but the larvae were not recorded from the sunk food bags. The correlation of larval loss in the effluent and fly abundance is close (+0·899), and there is negative correlation with the abundance of surface growth (−0·526).

The predacity tests revealed some carnivorous tendency as the larvae devoured the eggs of *Psychoda alternata* (7/7) and of *Spathiophora hydromyzina* (7/9), also the cocoons of *Lumbri-*

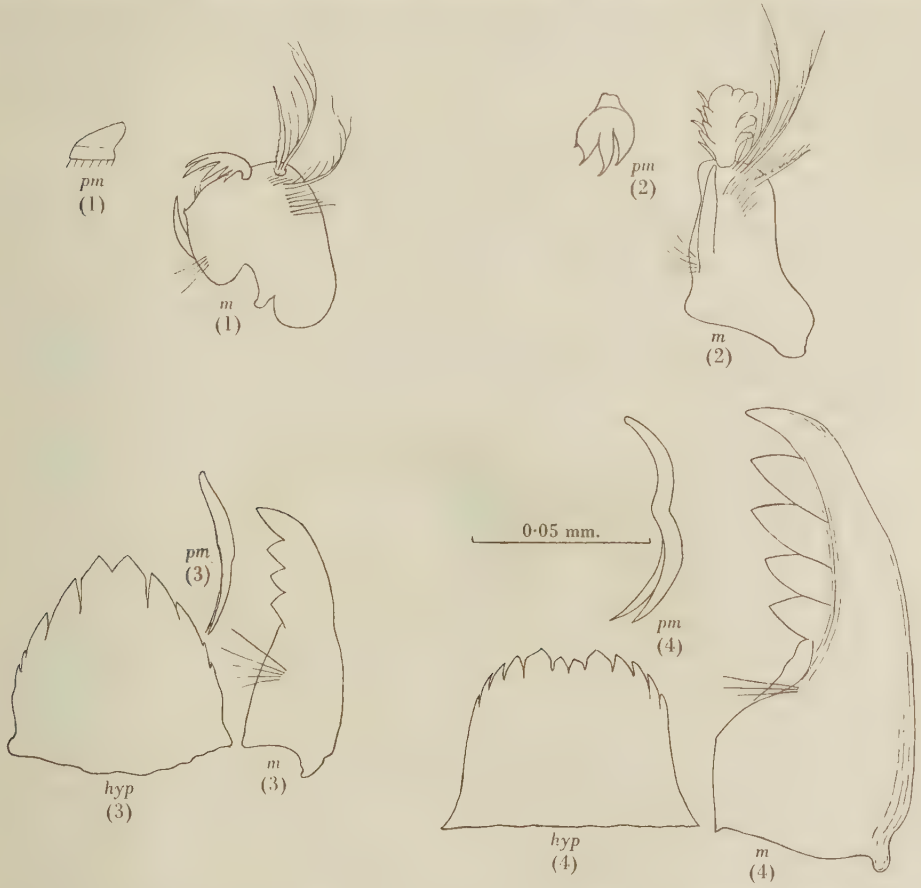


Fig. 1. Mouthparts of (1) *Psychoda alternata*; (2) *Psychoda severini*; (3) *Spaniotoma minima*; (4) *Metriocnemus longitarsus*. *m*, mandible; *pm*, premandible; *hyp*, hypostomal plate.

cillus lineatus (20/50) and the worms (4/32), also the larvae of *Spathiophora hydromyzina*, newly hatched (4/4) and old (1/5), also the pupae of *Psychoda alternata* (5/5). They did not eat the eggs of the chironomids (0/2) but destroyed their cocoons (10/12), eating only one of the pupae while the flies emerged normally from the other freed pupae. The mouthparts that enable them to make these attacks are figured in part (Fig. 1). The mouthparts of *Psychoda* larvae are very spinous. The labrum-epipharynx is strongly reflexed ventrally like a broad grapnel and carries two articulated sclerites, the premandibles of Goetghebur. The mandibles are rather quadrate and at the outer tip bear three feather hairs, one of which is serrate and

stiff: the inner tip carries an articulated palmate sclerite strongly spined, and this is opposed by a very strong spine originating near the base of the mandible. The palmate sclerite works against the tip of this rather as one can work the fingers against the opposed thumb. The body of the mandible has also several combs of spines. The maxilla is feebly developed and the hypostomal lobe is not dentate, at least in the forms observed. In *P. severini* the parts are more strongly sclerotized than in *P. alternata*, the premandible being a stout trident and the palmate sclerite like an outspread hand with the fingers slightly flexed. The gape between it and the opposing spine is greater than in the allied species and it seems these differences may account for the egg-eating proclivity of *P. severini* which is absent in *P. alternata*.

Psychoda alternata

This fly is not parthenogenetic. Mating takes place in or on the beds or on the walls, and the clustering habit brings the sexes together. Laboratory tests have proved that it will carry out mating and the whole life cycle in complete darkness. It lays some 100–150 eggs inserted singly or in small clusters into the food or naked on the pebbles which may be visibly whitened by them on occasion, so numerous do the flies become. The time of its cycle from 10° C. upwards agrees with a threshold of 6° C. and a thermal constant of 315° C. The following is typical of the behaviour in cultures, life-cycles and family size. The times are for females, males are a few days quicker, sexes about equal:

7° C. 140 days: 36 families, $2{\cdot}1 \pm 0{\cdot}2$, 25 failed, mortality mainly eggs and young larvae.
10° C. 80 days: 17 families, $26 \pm 7{\cdot}9$, 0 failed.
15° C. 35 days: at 17° C. 24 families, $40 \pm 6{\cdot}3$, 2 failed.
20° C. 22 days: 61 families, $71 \pm 4{\cdot}4$, 4 failed.
25° C. 16 days: 36 families, $86 \pm 7{\cdot}8$, 0 failed.
28° C. 14 days: 11 families, $80 \pm 6{\cdot}8$, 0 failed.
30° C. 13 days: 17 families, $57 \pm 7{\cdot}8$, 1 failed.
33° C. 5 attempts, 3 oviposited, 2 lots hatched, total mortality in late larvae and pupae.

The favourable zone is from about 15 to 30° C., but occasional large families have been obtained at 13° C. It is thus distinctly a warm-weather fly and is, to this extent, a complementary species to *P. severini* which is active at low temperatures, and whose early stages make progress even in an ice box at 1–2° C. The two breed at about the same speed at 12–14° C., and below this *P. severini* has the advantage, but at higher temperatures *P. alternata* draws rapidly away. The flies produced at the lower temperatures are larger insects.

The adult population is difficult to assess in the terms used for the other flies because its light reactions are so different. It seeks dusky places, and though in summer considerable numbers come into the light jars, far more remain in the trays below. These latter may total thousands and counting is impracticable, so a rough estimate based on a partial count is made at each weekly removal of the trays, and the numbers given in Table 2, col. 1, are averages of these. The correlations with the larvae strained out are based on these figures, as they are a fairer index of abundance than the jar trapping affords.

In a normal year, the flies appear on the bed surface in small numbers in March, and these rapidly increase to a peak output in May–June; there is then a decline, well recognized by the managers of sewage works and attributed to food shortage (Fair, 1934), and a second rise 1–2 months later with scarcity on the surface again in November. The mild winter of 1937–8 modified this behaviour, for a few were taken in the jars in each month and they were

rather abundant in the trays, increasing rapidly in February–March, the highest density of 1938 being in April.

The greatest concentration of the larvae in the beds is generally considered to be 3–12 in. below the surface (Fair, 1934), but this does not apply at Knostrop since the food bags at 2 ft. 6 in. in every month collected more larvae than did the bags at 1 ft., the aggregates being 3175 and 370 respectively. Also at Knostrop these larvae are rarely found in the trapping jars, but at Barnsley this was a common event. This difference is believed to be due to the occupation of the shallow zone at Knostrop by the dense population of *Metriocnemus* larvae, absent at Barnsley (p. 138).

The larvae and pupae are as likely to be dislodged by the water trickle as are those of *P. severini*, being of similar habit, and they appeared in the strainer in large numbers in May–June 1937 and again in March–April 1938. The numbers of larvae thus passing out show correlation with fly density (+0·699) and with the larvae in the food bag at 2 ft. 6 in. (+0·571), and also negatively with the surface growth (−0·563). The *Psychoda* pupae in the strainer, mainly of this species, correlate with the fly density (+0·775) and with larval density at 2 ft. 6 in. (+0·544).

In the predacity tests the larvae did not attack the eggs of *P. severini* (0/13) or *Spathiophora hydromyzina* (0/4), but they ate the mucus from the egg ribbons of the chironomids without reducing the subsequent hatch (6/8). They made no attack on the larvae of *Psychoda severini* (0/12) or *Spathiophora hydromyzina* (0/5). They destroyed the cocoons of *Metriocnemus longitarsus* (8/11) and three times ate the pupae thus freed and they also destroyed the cocoons of *Spaniotoma minima* (16 19) and seven times ate the pupae also. They did not attack the pupae of *Psychoda severini* (0/8) and damage to *Lumbricillus lineatus* was slight, worms (1/25) and cocoons (5/35) being devoured.

The carnivorous tendency is thus less than that of *Psychoda severini*, since they showed definite eating of the organism in only 16/132 tests (12%), while *P. severini* showed its carnivorous capacity in 49/126 tests (39%). This difference seems in keeping with the development of the mouthparts (Fig. 1) which are less sclerotized. The premandible is like a garden rake, while the palmate sclerite of the mandible is more spinous and like a half-clenched hand, the gape between it and its opposing spine being less than in *P. severini*. The structures are too minute and obscure to watch in action, but it seems such structures are less capable of gripping a slippery egg or worm cocoon.

Spaniotoma minima

This tiny black chironomid on the Knostrop beds rivals *Psychoda alternata* in abundance, and the adults have been taken in every month during five years' trapping. They form a mating swarm within a foot of the ground, but it is not a localized swarm as in many chironomids. It may stretch uninterruptedly along the whole of the dry parts of the terraces or the edges of the walls. They require calm and rather mild weather for mating, but it has been observed when the shade temperature was 10° C. They rarely mate in confined spaces, and with flies from culture in numerous attempts only one effective mating has been obtained. When the jars of trapped flies are brought to the laboratory they are first released into bell jars, 8 × 5 in., and left for 1 hr. When *Spaniotoma minima* is abundant a few will couple in the first mad rush out of the jar when they struggle in a heap at the lighter side, but thereafter no more matings occur however long they are kept. From 958 female flies tested for

fertility after this procedure only fifteen (1·5%) proved fertile. This mating bar no doubt holds the insect back in early spring when *Psychoda alternata* is mating in the warm darkness of the beds.

The fly lays 150–200 eggs in a single row in a mucous ribbon, and this forms a compact heap on the wet pebbles. The complete life cycle from 10 to 24° C. agrees with a threshold of 4·5° C. and a thermal constant of 450° C., the factors for female maturation having been found by keeping flies coupled in nature at the graded temperatures. In the families the sexes are about equal and emerge together, the males having a day or two advantage on the average. There is a good deal of time scatter in the emergence of a family, the quickest larva commonly taking about two-thirds and sometimes only one-half the length of time the slowest one takes for growth. The average complete life cycles and families observed in culture are as follows:

3° C. (1·6–6·5° C.)	260 days: 1 family of 5 flies: at 4–5° C. few oviposit and fertility of the eggs is poor, most larvae die young.
7–9° C.	103 days: 5 families, 69·21 ± 8·6, 0 failed, but there may be a mating bar.
10° C.	80 days: 7 families, 97·3 ± 9·9, 0 failed.
15° C.	43 days: 10 families, 103·5 ± 11·2, 0 failed.
20° C.	29 days: 13 families, 65·4 ± 10·5, 0 failed.
24° C.	22 days: 10 families, 10·0 ± 3·1, 0 failed.
25° C.	2 attempts, few pupae produced and these all died.

Fully favourable temperatures except for mating appear to be about 9–20° C. The fly has a steady monthly incidence with a minimum about February and a maximum usually in August.

The larva is active and strongly prehensile and seems to be distributed through the bed. At least it shows no climbing tendency and has no shallow concentration like that of *Metriocnemus* spp., and the numbers taken in the food bags at 1 ft. and 2 ft. 6 in. were about equal and both small. The numbers taken in the straining were also quite small (Table 3) and correlate with each indication of abundance (Table 6). It is of interest that of all the larvae and worms taken in the straining *Spaniotoma minima* alone gives a positive correlation with the amount of surface growth (+0·528), all the others giving negative ones. The mature larva makes a cocoon of frass or débris like a tiny mouse dropping fixed on to the food or pebbles. The pupa remains active and leaves the cocoon if disturbed. The cocoon is attractive to *Psychoda* larvae, and there is no doubt some loss in the beds due to this as the pupae have only feeble gripping power. In the straining, 231 pupae were taken to 138 larvae, and the duration of larval and pupal lives at summer temperatures are about 30 and 2·5 days respectively. Thus a far greater proportion of pupae than larvae are lost.

In the predacity tests the larvae showed a carnivorous tendency. They ate the hatching larvae of *Metriocnemus longitarsus* (1/1), the eggs of *Psychoda severini* (4/6) and of *P. alternata* (8/8), and the cocoons of *Lumbricillus lineatus* (18/20) and made slight attack on the worms (2/20). They also ate the larvae of *Psychoda alternata* nearly mature (17/23) and its pupae (6/6). They destroyed the cocoons of *Metriocnemus longitarsus* (8/11) and ate the pupae (3/11), and in a number of instances when the larvae of *Spaniotoma minima* have been isolated in small groups to determine pupation periods with the food purposely kept scanty to avoid obscurity they destroyed their own cocoons and ate the pupae. The premandible, the mandible and the strong hypostomal lobe, dentate and curved like a scoop, are illustrated in Fig. 1. They are evidently suited to the adoption of a carnivorous diet.

TABLE 3. *Record of larvae and pupae of* Metriocnemus longitarsus *and* Spaniotoma minima *counted in strainings of the bed effluent with corresponding indications of abundance, flies emerging on* 1 *sq. ft., larvae trapped in the depths. The first item and the last cover single weeks, the other items* 4 *weeks, averages*

	M. longitarsus					*S. minima*				
Dates of Strainings	Fly on surface ♀	Larvae at 1 ft. deep	Larvae at 2 ft. 6 in. deep	Larvae strained	Pupae strained	Fly on surface	Larvae at 1 ft. deep	Larvae at 2 ft. 6 in. deep	Larvae strained	Pupae strained
1937 22 Apr.	31	—	—	0	0	63	—	—	0	0
29 Apr., 6, 14, 20 May	14	—	—	2·5	3·2	97	—	—	0	0
27 May, 3, 10, 17 June	12	383	233	7·2	4·0	200	0	0	0·5	0
24 June, 1, 8, 15 July	4	82	0	5·7	6·5	425	0	0	1·0	0
23, 29 July, 5, 12 Aug.	9	86	2·3	1·0	1·0	1211	0	0	4·7	11·5
19, 26 Aug., 9 Sept.	3	62	2·0	0·3	0	1163	30	0	7·0	22
16, 23, 30 Sept., 7 Oct.	5	30	4·4	0·5	0·2	675	61	74	14·2	18
14, 21, 28 Oct., 4 Nov.	12	110	2·0	0·5	0	875	81	34	2·7	1·2
11, 18, 25 Nov., 2 Dec.	13	243	26	0	0	293	12	30	1·0	1·2
9, 16, 30 Dec.	38	345	9·5	0	0·3	292	23	16	2·3	2·3
1938 6, 13, 20, 27 Jan.	5	70	2·0	0	0	50	11	5	0	0
3, 10, 17, 24 Feb.	1	39	1·5	0	0	5	2	1	0	0
3, 10, 17, 24 Mar.	3	23	0·7	0	0	26	19	6	0·7	0·2
31 Mar., 7, 14, 21 Apr.	4	115	4·7	0·7	0	26	5	1	0·2	0·2
28 Apr.	8	35	2	0	2	18	12	0	2	8
Totals strained out				74	63				138	231

Metriocnemus longitarsus

These flies mate readily in confined spaces, in strong contrast to *Spaniotoma minima* and *Metriocnemus hirticollis*. They have been seen coupled in the small trapping jars and, amongst 390 females which had no more liberty than this and in the bell jars in the laboratory, 130 proved to be effectively mated. This capacity is of great advantage to a fly active in the depth of winter when long periods of wind deter the formation of swarms. Swarms are formed, however, in more genial weather and are rather compact, commonly above the ends of the bed walls or accompanying the travelling distributors.

The fly deposits about 450 eggs in two to three irregular rows in a mucous ribbon commonly disposed in several pieces. It is a low-temperature insect. The low limit for mating and maturation is not known, but the latter proceeds smoothly at 4° C. Other stages proceed without undue mortality at 1–2° C. with periods: incubation 31 days, pupation 26 days, and larval growth, not observed right through at 1–2° C., took 185 days at 2·8° C. (1·7–5·0° C.). Completed life-cycles were at follows:

2·8° C. 243 days (234–255).
6° C. 153 days (132–186).
10° C. 94 days (68–158).
16° C. 49 days (30–62).
18·5° C. 36 days (29–41).
21° C. 26 days (25–29).

The sexes are about equal and occupy about equal time. Viability in all stages is good from 5 to 18° C., there is great larval mortality at 20° C. and no pupae have survived above 21° C. The series given above is not fitted by a thermal constant formula, but evidently the actual threshold is in the neighbourhood of 1° C. The variation in the speed of larval growth in the single family is remarkable and is due to intermittent feeding. There is an evident advantage to these chironomids in the scatter of emergence which avoids the possibility of all the individuals of a brood emerging during a spell of unfavourable weather. This is particularly useful for a species that has its maximum flight in winter.

The larvae have a strong tendency to climb, and this is based on a dislike to prolonged submergence, though they will submerge to feed and can pass all early stages without access to air if in well-aerated water (Lloyd & Turner, 1936). They concentrate in the upper 1 ft. of the beds and pupate just below the surface (Dyson & Lloyd, 1936). Evidence of this is given by the larvae trapped in the food bags, 5647 in the 1 ft. bag but only 1077 (932 in one week) at 2 ft. 6 in. (Table 3), reversing the distribution of *Psychoda alternata*. The cocoon is flask-shaped, open at the head end, and is of clear mucus. There is a strong tendency to cluster, ten or more being often packed closely.

The flies emerge from the beds the whole year through and have been recorded in every month over five years. The heaviest emergence has always been November January, and there is a lower peak period about May. Summer conditions are adverse, because even such a short rest as the distributor gets for cleaning, causes in dry weather some drying and heating of the surface zone where the pupae lie, while on hot days with the machine working the temperature may rise to 21–22° C. below the surface pebbles (Lloyd, 1937).

The larva is strongly prehensile and vigorous and its bulk is about five times that of *Spaniotoma minima*. Very few were strained out of the effluent, the aggregate being only seventy-four larvae and sixty-three pupae. As the pupal life is only some one-tenth to one-twentieth that of the larva the pupal loss is relatively heavier, as with *S. minima*. These small numbers are far from correlation with the fly output (larvae −0·106, pupae −0·095) and do not agree with the catches of larvae (+0·437), but they do correlate with the solids (larvae +0·819, pupae +0·950) and negatively with the surface growth (larvae −0·704, pupae −0·670).

In the predacity tests the larvae ate the eggs of their own species (5/6), of *Spaniotoma minima* (6/8), of *Psychoda alternata* (9/10), of *Psychoda severini* (18/18) and of *Spathiophora hydromyzina* (4/4), also the cocoons of *Lumbricillus lineatus* (19/20) and some worms (10/30). They ate the larvae of *Psychoda* (4/8) and *Spathiophora hydromyzina* (3/5). They also ate *Psychoda* pupae (6/16) and *Spaniotoma minima* pupae (3/9) while they destroyed their own cocoons (4/4) and those of *S. minima* (6/9). In this series they did not eat their own pupae though this cannibalism has been observed (Lloyd, 1935). They have thus the strongest carnivorous tendency amongst these larvae, and the mouthparts are well adapted to making these attacks, resembling closely those of *S. minima* but larger and more powerful correlative with the larger size of the insect.

Metriocnemus hirticollis

These flies seem incapable or almost so of mating without a swarm. In the bell jars of the laboratory they very rarely couple and amongst 147 females taken from these only three laid fertile eggs (1·5 against 30% with *M. longitarsus*), and this small proportion cannot be

regarded under the circumstances as certainly due to mating in confinement. Though the light-coloured females are readily distinguished from the black allied species, the males are so much alike that we are unable to distinguish them and this makes a study of the mating swarms difficult when both species are in flight. Couples of both species are to be found below what appear to be homogeneous swarms, so the males may mix in flight. *M. hirticollis* is less regular in its appearance than are the other species dealt with, and this may be because of difficulties encountered in the formation of mating swarms. Though their temperature relationships mark them as warm-weather flies, the warm conditions of the beds bring them out in winter and they have been absent from the trapping jars in only 1 month in the long record, the very cold February of 1936. There has always been a distinct though low peak in September–October and generally a higher peak in April–June, while great abundance was observed in January–February 1935 following a favourable close to 1934. In the year of the straining tests they were never abundant and this period followed an almost complete eclipse in the winter of 1936–7, when in 6 months the three traps yielded a total of only eighteen females.

The adverse effects of cold are shown in the following figures. At 5° C. egg development was partial, hatching in 13 days, while eggs of the same flies gave normal hatching at higher temperatures. At 5° C. also most of the young larvae died while the survivors made little progress and after 90 days were still minute, hardly larger than at eclosion, but they matured at favourable temperatures. Pupation, however, can proceed at 3° C. (15-18 days, 6/10 surviving). Completed life cycles were as follows:

10° C. 123 days (107–139).	17° C. 37 days (27–46).
15° C. 49 days (40–58).	20° C. 42 days (23–51).

10–20° C. are favourable, 25° C. is too high, the larvae dying off during growth but some near maturity. Except for the lag at 20° C. the series is about satisfied by a threshold of 6·7° C. and a thermal constant of 400° C. Like the two *Psychoda* these two *Metriocnemus* are thus functionally complementary species.

As remarked, during the year of straining the insect was relatively not abundant in the beds, and none of its larvae was observed in the straining or recorded from the food bags; its larvae are quite distinctive in this assemblage being uniformly cream-coloured, while those of *M. longitarsus* are purple ringed. They and the pupae are in habit and location as those of *M. longitarsus* and would be expected to suffer a proportionate loss in the effluent.

Their predaceous tendency seems similar to that of the allied species, and the mouthparts differ in small detail only. They destroyed all their own cocoons and those of *Spaniotoma minima* to which they were given access and ate the pupae of *Metriocnemus longitarsus* (4/6) and of *Spaniotoma minima* (4/5).

Spaniotoma perennis

In the strainings sixty-one larvae and thirty-two pupae of this fly were taken, all but one larva from May to August. This is not often a common fly on the Knostrop beds, and during the relative 12 months of trapping only eighty flies (twenty-six to 1 sq. ft.) were taken, fewer than the larvae and pupae washed out. This is in strong contrast to *Metriocnemus longitarsus* (1040 flies to 1 sq. ft., 138 larvae and pupae strained) and *Spaniotoma minima* (22,000 flies to 1 sq. ft., 371 larvae and pupae strained out). The reason for this difference lies in the habit of this insect in going downwards to pupate, and it alone in this assemblage of

nematocerous flies has shown a diapause in laboratory breeding. It has been observed in culture that when the larva is fully fed it commonly burrows below the cotton-wool foundation in its jar. One culture was kept at 7–9° C. from hatching on 27 January till 26 June, when the larvae had ceased to feed and had burrowed. They were then transferred to room conditions and treated in a variety of ways to induce pupation but without effect till October–November, when pupation took place without feeding, and four male and seven female flies were produced. This prepupal diapause developed also in a few individuals from January cultures kept at higher temperatures from eclosion, and there is strong evidence that it is universal in nature in summer, since the fly has not been taken during three years in August or September, only two in July, three each in October and November, whereas 713 have been recorded from December to June. Not enough data are available to define its temperature relationships, but it is distinctly a cool-weather insect, and it mates in confined spaces, since impregnated females are readily obtained among the trapped flies. No doubt the burrowing habit of the larva with consequent loss in the effluent is the limiting factor that prevents it from being a successful insect in the rather open medium of the Knostrop beds. It has been observed (Lloyd, unpublished) in vast numbers on some choked beds in Cheshire, where it made itself a pest in a neighbouring house. Its predaceous capacity has not been studied.

Spathiophora hydromyzina

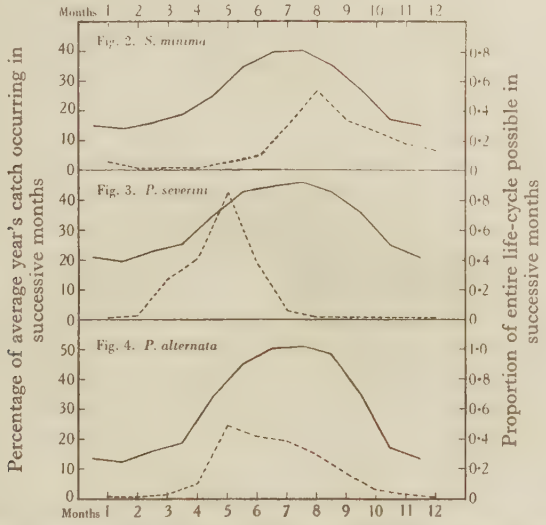
This is the only member of the higher Diptera that has colonized the beds. The fly is abundant from spring to autumn, when it is to be seen stalking and catching *Psychoda* and the chironomids, and when one enters the trapping jar it causes much destruction. However, it is not often so taken, perhaps because the larva tries to reach the drier parts of the beds, the edges or ends, for pupation. The larva is to be found near the surface of the bed, and it grazes over the pebbles. It has been observed under a lens apparently eating the clusters of *Lumbricillus lineatus* (Lloyd, 1935), but laboratory tests do not show it to be specially carnivorous. The cocoons of *Metriocnemus longitarsus* (6/6) and the pupae (2/6), also the cocoons of *Spaniotoma minima* (3/3) and the pupae (1/3) have been eaten, but not the eggs of these flies or of *Psychoda* (0/6, 1 case doubtful). It showed less attack on *Lumbricillus lineatus* than the other larvae tested, eating cocoons (1/30) and the worms (2/50).

Evidence of the competition

This evidence is on three lines: (1) the occurrence of deviations in the seasonal abundance of some of the insects from what might be expected; (2) the effects of differences in the compositions of the fauna at the Knostrop and Barnsley Works on incidence and abundance of the flies; (3) the effects of differences in the relative abundance of the flies on various parts of the Knostrop beds.

Fly trapping has been uniform and almost uninterrupted at Knostrop during five years. In each year the numbers of a species taken in each month have been expressed as percentages of the total of that species taken in the year. These percentages plotted against the months give a curve of incidence for the year. (For one month and three shorter periods when no trapping was done the numbers were deduced from adjacent periods and interpolated.) The five curves of monthly incidence were then combined into a single average curve of incidence for the species. The mean monthly bed temperatures were also averaged

and with these, together with the data given above for speed of life cycle at graded temperatures, it was possible to define a token factor of progress for each month of the year. This is expressed as that proportion of the whole life-cycle the insect could achieve in 30 days if food was adequate. These token numbers, plotted against the months, give what may be called curves of expected progress. When these curves are low and flat a low curve of incidence would be expected, but, following a rise, there should be increasing incidence with an apex after the highest point of the progress curve, provided always that food was adequate and no adverse circumstance developed. The curves should then decline more or less parallel to each other. The two *Psychoda* spp. and *Spaniotoma minima* lend themselves to this treatment as there is nothing adverse to them in the climate of the beds except the normal retarding effect of winter cooling. *Metriocnemus* spp. cannot be dealt with conveniently in this manner because of the summer depression caused by their habit of feeding and pupating near the bed surface where spells of drying are adverse.



Figs. 2, 3 and 4. Seasonal incidence of *S. minimi*, *P. severini*, and *P. alternata* contrasted with their curves of expected progress. Averaged on five years' records. Incidence: broken line; progress: continuous line.

Spaniotoma minima (Fig. 2) most nearly follows expectation but seems slower in its spring rise than might be possible. Although the fly is dependent on rather mild weather for mating, suitable days do occur in December and January when the flies are emerging in fair numbers. From these, in theory, two generations could pass by mid-June, but the usual June abundance is lower than that of December. Two facts may bear on this. First, up to June *Psychoda* spp. are approaching their zenith and *Metriocnemus* spp. are also very abundant prior to their summer decline. Secondly, the offloading of the beds brings food to a minimum in late spring. From June onwards food tends to increase, *Metriocnemus* for 2 or 3 months is relatively scarce, and *Spaniotoma minima* in its turn becomes the dominant insect in the beds. It reaches its zenith in August, as the curve of expected progress suggests it should do, but it declines rather rapidly in the early autumn in spite of genial mating days. At this time *Metriocnemus* spp. are increasing swiftly.

Psychoda severini (Fig. 3) starts from very low numbers at the start of winter at Knostrop but could achieve two generations by mid-May. Its numbers increase rapidly according to expectation, but the very abrupt form of curve resulting is of course due to its almost total eclipse from July onwards, though the insect should be doing well the year through in theory, nearly eight generations being possible.

Psychoda alternata (Fig. 4) follows its curve of expected progress at the beginning of the year and reaches its peak in May when two generations could have passed. Nearly five more generations are possible in theory, but it declines rapidly away after July. The well-known depression in early summer (p. 130) is only just indicated in this compound curve of incidence because it is a shifting point and in any case is not so distinct as a winter decline. The almost complete depression in mid-winter is as expected, but as the fly can continue to mate in complete darkness it may go on breeding slowly in the depths of the bed without showing at the surface.

Decline of both species of *Psychoda* after the spring abundance is to be expected because the spring offloading of the beds leads to a temporary shortage of food. No reason other than interference can be suggested for the failure of *Psychoda severini* to recover and for the rapid second decline of *P. alternata* in the later summer. It is to be noted that during the later summer *Spaniotoma minima* is working up to its zenith, and that when this species in turn comes to its rapid autumn decline, *Metriocnemus longitarsus* is in its turn working up to a zenith, conditions on the beds becoming very favourable to it.

The evidence of the competition afforded by comparisons of the fly output of the Knostrop beds with those of Barnsley has been given by Lloyd (1935, 1937) and will be briefly recalled. At Barnsley *Metriocnemus* spp. are absent and their absence is attributed to the system of long rests given to the beds as these would naturally be adverse to the early stages. *Spaniotoma minima* is present at both places, and in a two years' record no important differences in its total output were observed, but at Barnsley it is seasonally a later fly reaching its zenith output in September–November, as against August–September at Knostrop. This difference may also be due in part at least to the summer rests as the fly oviposits largely just below the surface, but it may also be partly due to less interference in autumn where *Metriocnemus* is absent. The *Psychoda*, on the other hand, are much more abundant at Barnsley and the summer depression of *P. severini* is far less pronounced, with the result that it is emerging there in great numbers in mid-winter when its increase is only just perceptible at Knostrop. For exactly the same amount and routine of trapping during 2 years at the two works the output of *Psychoda* on 2 sq. ft. was: Knostrop, *P. alternata* 2913 and *P. severini* 16,032: at Barnsley, *P. alternata* 27,834 and *P. severini* 67,717. *P. alternata* is really the more abundant fly at both places, but the different light reactions of the two species obscure this point as explained above (p. 130). The rough estimates of the tray accumulations of *P. alternata* which give the best evidence of abundance were not kept at Barnsley.

More exact evidence of the competition has been obtained by trapping on different parts of the Knostrop beds. The sewage distributor takes 30 min. for its double journey over the bed, and so near the end it spreads its sheet each $\frac{1}{2}$ hr. but at twice this frequency in the middle. This falling sheet of water disturbs the flies and the distributor acts rather like a brush tending to accumulate them at the ends of the traverse. Also the trickle must disturb the flies in the act of ovipositing, and twice as often in the middle as at the ends. This action does not affect all species equally and, with *P. alternata* in particular, its effect is not even

throughout the year as this fly is loath to take to flight except in warm weather, and, only under such conditions, are accumulations of them found on the end-walls. This disturbing effect is also subject to irregularities because during short stoppages of the distributor, which are frequent for oiling and cleaning, the insects are given an opportunity to oviposit without disturbance. Only long record of trapping could reveal this influence. Three such records are available, and in each of these one trapping site was about 4 yd. from the end of the bed and the contrasting traps were placed respectively at 10, at 15 and at 35 yd. from the end, the last being in the middle of the traverse. At the end there is the low supporting wall of the bed and the raised channels with large inflow pipes which afford the favourite shade for *P. alternata* in hot weather, while above the ends of the channel walls the densest mating swarms of *Metriocnemus* occur. The densest mating swarms of *Spaniotoma minima* form over the narrow strip of medium which the sprayer does not reach. The mated couples of the chironomids drop on to the wall or inflow pipes, and the females mature especially just below the surface of the dry medium. A grassed bank beyond the wall is also often the site of dense *S. minima* swarms. It is to be understood that these things are comparative, as swarms may be seen elsewhere and even accompany the top hamper of the distributor in the case of *Metriocnemus*.

Two of the three records of this differential trapping are analysed in tables showing the monthly totals of the various species at the contrasting positions, the total number of collections made, and the number of times that the flies were in excess in one or other of the traps, equal in both, or absent in both. Tables 4*a* and 4*b* give a record from February to December 1937 when the traps were at distances of 4 and 15 yd. from the end but on adjacent beds. Tables 5*a* and 5*b* give a record when the traps were on one bed, 4 and 35 yd. from the end, period January–December 1938. (The third record of this type when the traps were only 6 yd. apart is not recorded in detail but it holds nothing contrary to the conclusions drawn.) In Table 5 under *Psychoda alternata* is given a record for "light and dark traps", the former referring to the collections from the glass jars and the latter to collections from tins, 4 in. deep and $2\frac{1}{2}$ in. diameter, containing a crumpled cloth and sunk into the bed near the tray. For convenience the records are placed together. These tins were also invaded by *Metriocnemus* larvae which pupated there, and comparative counts of these are available from April onwards.

These tables should be consulted in relation to the following considerations which apply to both of them:

(1) *Spaniotoma minima* tends to be more abundant nearer the middle of the bed in the earliest months of the year, but it does not reach a high output there till July or August. Its output at the end of the bed becomes greater than that at the middle in spring, and the fly reaches a high output in June or July; the marked excess at the end thus established is generally maintained till December. The marked increase near the end of the bed (June or July) about coincides with the summer decline of *Metriocnemus*.

(2) *Metriocnemus* males and *M. longitarsus* females are respectively more abundant at the end of the bed in 20 and 19 out of the total 23 monthly records given. The important exception November–December 1938 is discussed below. *Metriocnemus* larvae and pupae were far more abundant at the end in each month of their record and in 32/34 of the individual collections. *M. hirticollis*, recorded by females as the males are included above, seems more abundant towards the middle in both these records, as well by total captures as by

TABLE 4*a*. *Analysis of Diptera trapped on* 1 *sq. ft. of bed,* 4 *and* 15 *yd. respectively from the end of the bed. Totals caught by months*

1937	*S. minima*		*Metriocnemus* ♂		*M. hirticollis* ♀		*M. longitarsus* ♀		*P. severini*		*P. alternata* light traps		*P. alternata* rough estimate	
Feb.														
4 yd.	46		13		0		9		10		2		40	
15 ,,		187		6		2		6		37		2		70
Mar.														
4 yd.	56		29		0		26		1		0		1	
15 ,,		242		14		1		9		30		0		25
Apr.														
4 yd.	141		93		0		65		46		25		350	
15 ,,		76		37		0		19		260		23		1000
May														
4 yd.	276		268		1		67		252		1228		1300	
15 ,,		511		114		8		77		611		521		1550
June														
4 yd.	816		154		2		98		59		389		1175	
15 ,,		225		20		9		17		180		298		1100
July														
4 yd.	6162		82		23		42		12		121		500	
15 ,,		1093		23		3		7		20		214		625
Aug.														
4 yd.	9822		39		6		33		0		98		525	
15 ,,		4400		17		4		9		2		190		1550
Sept.														
4 yd.	5205		52		10		19		0		40		1050	
15 ,,		2567		52		16		27		0		172		2550
Oct.														
4 yd.	3879		56		9		30		0		31		600	
15 ,,		2920		37		14		6		0		39		550
Nov.														
4 yd.	2900		162		10		86		0		12		2800	
15 ,,		2077		82		20		61		0		9		1275
Dec.														
4 yd.	1672		413		16		322		0		4		300	
15 ,,		763		74		18		78		1		1		270
Totals														
4 yd.	30975		1361		77		797		380		1950		8641	
15 ,,		15061		476		95		316		1141		1469		10565

TABLE 4*b*. *Analysis of trapping detailed in Table* 4*a by a number of collections showing excess*

Insect	Total collections	Excess at 4 yd. in	Excess at 15 yd. in	Equal in	None present
S. minima	89	51	36	1	1
Metriocnemus ♂	89	62	18	4	5
M. hirticollis ♀	89	20	28	5	36
M. longitarsus ♀	89	54	23	7	5
P. severini	89	5	36	1	47
P. alternata:					
In light traps	89	23	40	3	23
Rough estimates	39	15	16	7	1

TABLE 5*a*. *Analysis of Diptera trapped on 1 sq. ft. of bed, 4 and 35 yd. respectively from the end of the bed. Totals caught by months*

1938	*S. minima*		*Metriocnemus* ♂		*M. hirticollis* ♀		*M. longitarsus* ♀		*Metriocnemus* larvae and pupae*		*P. severini*		*P. alternata* light and dark traps*		*P. alternata* rough estimate	
Jan.																
4 yd.	135		19		0		23		—		1		3		175	
35 „		110		13		0		16		—		4		22		1200
Feb.																
4 yd.	52		23		0		9		—		2		2		610	
35 „		76		3		3		3		—		28		14		3750
Mar.																
4 yd.	55		43		0		42		—		9		61		2800	
35 „		69		3		3		5		—		213		84		6000
Apr.																
4 yd.	150		68		1		35		1063		174		615		17500	
35 „		25		2		2		1		11		284		644		10000
May																
4 yd.	480		50		0		46		217		39		460		8100	
35 „		42		7		7		3		29		1114		312		4300
June																
4 yd.	1602		54		9		48		571		17		309		3300	
35 „		92		10		10		8		69		643		301		1100
July																
4 yd.	1720		7		2		4		97		19		113		370	
35 „		265		10		2		13		82		280		203		600
Aug.																
4 yd.	4443		23		15		13		111		2		199		330	
35 „		2389		6		5		4		25		10		216		480
Sept.																
4 yd.	3237		47		16		40		486		1		216		2180	
35 „		1952		18		10		14		135		3		717		4250
Oct.																
4 yd.	1586		41		5		44		354		0		59		293	
35 „		2589		33		5		26		65		16		191		940
Nov.																
4 yd.	3126		105		8		66		824		1		29		285	
35 „		2339		263		37		169		506		27		249		5200
Dec.																
4 yd.	1742		324		11		302		687		0		7		200	
35 „		1924		331		24		482		96		22		34		2400
Totals																
4 yd.	18328		804		67		672		4410		265		2073		36143	
35 „		11872		699		108		744		1018		2644		2987		40220

* Trapped in tin, p. 139.

TABLE 5*b*. *Analysis of trapping detailed in Table 5a by number of collections showing excess*

Insect	Total collections	Excess at 4 yd. in	Excess at 35 yd. in	Equal in	None present in
S. minima	95	64	26	4	1
Metriocnemus ♂	95	59	24	5	7
M. hirticollis ♀	95	17	28	6	44
M. longitarsus ♀	95	51	24	7	13
Metriocnemus larvae and pupae	34	32	2	0	0
P. severini	95	5	62	1	27
P. alternata light and dark traps	128	38	69	6	15
P. alternata rough estimate	45	8	32	5	0

individual collections, but the numbers are small and rather erratic and too many records were by single flies. In the third record of this type of trapping (at 4 and 10 yd. from the end) in eighty-three contrasting collections *M. hirticollis* was more abundant nearer the end in forty-one collections, more abundant nearer the middle in sixteen collections, equal in three, absent in twenty-three, with totals at 4 yd. of 469, and at 10 yd. of 193, i.e. it behaved like the allied species when it was an abundant fly, so its distribution in its years of scarcity should be discounted unless further work explains the difference.

(3) *Psychoda severini* very definitely emerges in greater numbers nearer the middle of the bed. Its abundance tends to be inverse to that of the chironomids. It increases in spring at the end of the bed more slowly than nearer the middle; its decline near the end in early summer is more rapid and its eclipse is more complete, especially in 1938. When the monthly output of *Spaniotoma minima* is more than 1000 per sq. ft. the output of *Psychoda severini* becomes very small.

(4) *Psychoda alternata* is more abundant towards the middle of the bed till March or April. Then as warm days become more frequent there is an increasing tendency for the flies to gather at the end of the bed because they become more subject to the sweeping action of the machine as their flights become bolder. There follows a phase when it emerges from the bed in greater numbers near the end, and this phase continues to the end of June. The accumulations on the end-walls persist right through the summer, but, in spite of this, breeding, as evidenced by the trapped flies, is more successful nearer the middle of the bed from July onwards, November–December 1937 forming an exception to this general rule. That is, its tendency to breed more abundantly near the end of the bed in summer is frustrated when *Spaniotoma minima* approaches its zenith in July, the latter fly being more abundant there than in the middle.

(5) To summarize this section of the evidence for the competition, if the sweeping action of the distributor and the depressing effect of cold on the flights of *Psychoda* are considered, it would be expected that in the cooler months of the year oviposition of these flies should be fairly even over the bed, but in the warmer months it would be greater nearer the end than towards the middle. If food was adequate, emergence of the flies should be proportional to oviposition provided there was no interference with the early stages of the insects. However, with the exceptions noted, *Psychoda* tend to emerge more abundantly nearer the middle of the bed, and the conclusion is drawn that there is some interference. The temperature effect on flight does not influence the chironomids so much because they must come into the light for mating. Therefore, owing to the sweeping action of the machine, heavier oviposition nearer the end would always be expected, and the general evidence drawn from the numbers of emerging flies is that this is so. The conclusion drawn is that the chironomids are interfering with the *Psychoda* either by depriving them of food, or by direct attack, or by both.

Evidence that there may be competition by direct attack is dealt with below (p. 143). Evidence of the competition for food is now being collected and is based on measurements of the size and weights of the emerging flies. This will be given in a future communication, but it has some bearing on the anomalous greater abundance of *Metriocnemus* nearer the middle of the bed in November–December 1938, Table 5*a*. In these two months the middle trap took the greater number of flies in twelve out of sixteen collections with totals, middle 1306, end 816. The larvae and pupae trapped, however, were more abundant at the end than in the middle in seven out of eight collections with totals, middle 602, end 1511, i.e. in the

middle the trapped flies totalled twice the trapped larvae and pupae, but at the end they were only half as numerous. This suggests that oviposition had been heavier near the end as usual but there was more loss. Six weighings of *M. longitarsus* females were made during this time, and on each occasion those near the end were lighter than in the middle. The mean weights were, g./1000 flies, end $1{\cdot}078 \pm 0{\cdot}0128$, middle $1{\cdot}230 \pm 0{\cdot}0277$.

Spaniotoma minima females were also being weighed from the two positions and fourteen comparisons were made from October to December. In all but one of these the flies from the middle were heavier than those from the end, the average weights, g./1000 flies, being at the end $0{\cdot}256 \pm 0{\cdot}0340$, at the middle $0{\cdot}286 \pm 0{\cdot}0388$. These differences are not significant as they stand because the great variation from week to week makes the standard error so great, but the average percentage excess weight of the flies from the middle over those from the end was $11{\cdot}7 \pm 2{\cdot}9\%$. There is thus no doubt that the *S. minima* larvae near the middle were being better nourished at this time than were those at the end of the bed, just as were those of *Metriocnemus*. Since all parts of the bed receive the same amount of tank effluent, the end of the bed must have been more populous with the grazing organisms than was the middle part.

When competition for food is under discussion some reference to the enchytraeids should be made. During the later part of 1938 only one of the authors was making observations and it was not practicable to continue every type of record, but in June–July, counts of *Lumbricillus lineatus* in 20 g. of surface alga were made at both trapping sites on four occasions. At the same times the dry weight of the surface alga on 1 sq. ft. of bed was found. The results were as follows: end site, weight of growth averaged 2·25 g., *L. lineatus* averaged 1550; middle site, weight of growth averaged 4·62 g., *L. lineatus* averaged 1200. There is no *prima facie* reason why the worm population should vary along the length of the bed, but if the population was uniform it would be expected that where the preferred food, the surface alga, was less the aggregation of worms in it would be greater than where the preferred food was more abundant. These results are therefore not discordant with a more or less uniform worm population. The indication that the surface growth was less near the end than towards the middle was borne out by surface inspection through all the later months of 1938, and this is further confirmation that the grazing organisms were actually more numerous nearer the end of the bed.

Discussion

The evidence given in the last section shows that the seasonal abundance of the flies is governed partly by the relationship of temperature to their rate of progress and well-being, partly by the supply of food which is regulated by the density of the population, and partly by what has been termed "interference". This interference might be by direct attack or it might be due to the activities of the larvae and worms loosening the attachment of the organisms to the food or medium.

Direct evidence of attack is hard to collect in the beds, and reliance has to be placed on the laboratory tests of predacity. These tests were made in the absence of other food and gave the following results: actual devouring of the organism was shown by *Metriocnemus longitarsus* in 87/134 (65%) cases, by *Spaniotoma minima* in 59/95 (62%) cases, by *Psychoda severini* in 49/126 (39%) cases, and by *P. alternata* in 16/132 (12%) cases. Thus the chironomids are more prone to adopt the carnivorous habit than the *Psychoda*, and of the latter *P. severini* is

definitely more carnivorous than *P. alternata*. These differences have been shown to be in keeping with the development of the mouthparts (Fig 1). This potential predacity is certainly a factor that must be reckoned with, for after the time of offloading normal food is scarce all over the bed, and, at other times, it becomes scanty locally as shown above (p. 143). Further, the eggs of *Psychoda* are inserted in food when this allows, and when the chironomid larvae encounter these they simply eat them as they graze.

These results are sufficient to explain why *P. alternata* and *P. severini* are less abundant at Knostrop than at Barnsley where there is no *Metriocnemus*. They also explain why there is a tendency for the local abundance of *Psychoda* on the Knostrop beds to be more or less in inverse ratio to the abundance of the chironomids. They have also an important bearing on an interesting point in the zoning of aggregations of the larvae in the beds. Excavation of bacteria beds has shown that *Psychoda* larvae are found throughout the depth but in greatest abundance 3–12 in. below the surface (Fair, 1934). Observation on the beds in the Leeds district where *Metriocnemus* is absent confirms this general rule. At Barnsley they are abundant near the surface and often invaded the trapping jars in numbers, a thing not observed at Knostrop. At Shipley they are found on every surface stone. At Huddersfield both *Metriocnemus* and *Spaniotoma minima* are absent, and Mr W. H. Golightly, who has made records there from food bags sunk to depths of 1 and 3 ft., states that the larvae of both *Psychoda* are abundant at the shallower level but relatively scarce at the deeper one. At Knostrop, however, in the long record of larva trapping by sunken food bags 3007 *P. alternata* were taken at 2 ft. 6 in. but only 484 at 1 ft. (Table 2). *Metriocnemus longitarsus*, on the other hand, gave 5647 at 1 ft. and only 1077 at 2 ft. 6 in. (Table 3). There is thus in depth that same tendency to the inverse abundance of these forms that is revealed by the local trapping of the flies. The impulse that brings *Metriocnemus* larvae near the surface has been mentioned above (p. 134), and there appears no reason why *Psychoda alternata* should be relatively scarce in its usually favoured zone except the abundance of the rival larvae with their carnivorous tendency. The favoured zone of *Lumbricillus lineatus* is also the top 1 ft., but it descends when food is scanty, and the numbers taken in the two food bags are not materially different (Table 1). The worm would not incommode the other grazers except as a competitor for food or by loosening the food on the medium. *Metriocnemus*, however, might well reduce *Psychoda* by predacity in the favoured zone, and the result would be that the greatest density of *Psychoda* larvae would lie deeper in the bed while the fly output would be less, because the volume of the medium where breeding could be productive would be progressively reduced according to the numbers of *Metriocnemus*.

It is not easy to separate the influence of *Spaniotoma minima* from that of *Metriocnemus longitarsus* on *Psychoda* numbers when both abound in the bed. No zone of concentration of *Spaniotoma minima* larvae has been found. It certainly does not congregate specially near the surface, and the numbers taken in the food bags were about equal and both small (Table 3). Direct evidence that it reduces the numbers of *Psychoda* is therefore not forthcoming, and the belief that it does so is based on the fact that it takes to predacity readily in the laboratory, and the evidence from fly trapping given above, viz. that in the time of summer depression of *Metriocnemus* it becomes the dominant fly on the Knostrop beds; that *Psychoda severini* which could then produce almost a generation a month fails to recover from its early summer decline; that *P. alternata*, though it recovers from its early summer decline, remains almost uniformly scarcer in those parts of the beds where *Spaniotoma minima* is breeding most

freely. In a straightforward struggle for food *Psychoda alternata* should at least keep pace with *Spaniotoma minima*, since its life-cycle is shorter at summer temperatures. Another instance of the apparent effect of *S. minima* on *Psychoda severini* is in the Barnsley record of 1934–5 (Lloyd, 1935). During the winter on three beds *Spaniotoma minima* had its usual winter scarcity and *Psychoda severini* had the winter abundance characteristic at these works. On a fourth bed *P. severini* was very abundant in January and *Spaniotoma minima* scarce, but the latter suddenly increased through February and March while *Psychoda severini* correspondingly declined. The effect could hardly be due to competition for food alone, but it could have been due to direct attack.

Judging by the tests of predacity, *P. alternata* can only make a slight counter-attack on the competing organisms, but that of *P. severini* might be more significant, especially in reducing the eggs of the flies and the cocoons of the worms.

There is conclusive evidence derived from observation on the beds that the loosening effects of one organism on another are important in the competition. Most of the oviposition is near the surface where *Lumbricillus lineatus* and *Metriocnemus* larvae most abound. *Lumbricillus lineatus* pulverizes the surface growth (Reynoldson, 1939) and so must set free many eggs deposited in it and these would be washed down. The predacity tests showed that the eggs of the chironomids are liable to be scattered by larvae. This occurred in 18/30 tests, the eggs themselves being eaten in 11 instances. The tests also showed that the cocoons of these flies are likely to be eaten and the almost helpless pupae thus set free. This occurred in 60/75 tests, the freed pupae being eaten in twenty-three instances. Both species of *Psychoda* and *Spathiophora hydromyzina* are adept at this cocoon destruction as well as the chironomids. The pupae of *Psychoda* are commonly lodged in food when this is bulky and must be freed when support is devoured. Many of the pupae thus set free may be held in crevices of the medium and, though at a disadvantage, some might survive, but many also must be washed to the bottom of the bed and out into the channels, a fate which must certainly overtake any loosened eggs that are not devoured. Some evidence of the loss of pupae was obtained in the strainings of the bed effluent.

The totals of organisms counted in these strainings were as follows: *Psychoda severini*, 675 larvae; *P. alternata*, 3782 larvae; *Psychoda* pupae, 734; *Metriocnemus longitarsus*, 74 larvae, 63 pupae; *Spaniotoma minima*, 138 larvae, 231 pupae; *S. perennis*, 61 larvae, 32 pupae; *Lumbricillus lineatus*, 3471 worms, 178 cocoons. There were fifty strainings of 5 min. duration, and only a fraction of the effluent passing was actually strained. It is therefore evident that the loss of *Psychoda* larvae and pupae and of *Lumbricillus lineatus* is great, while that of *Spaniotoma perennis* is catastrophic because of the burrowing habit of the larvae (p. 136). The proportionate loss of *Metriocnemus longitarsus* and *Spaniotoma minima* is less, and the loss of pupae in relation to larvae is decidedly greater than appears for reasons given above (pp. 134 and 132).

Inspection of Tables 1–3 shows that the heavy discharge of larvae, pupae and worms from the beds is mainly a spring phenomenon occurring about the time of the off-loading when solids in the effluent are near the maximum and surface growth near the minimum. *S. minima* is exceptional, but it is not then an abundant insect. The coefficients of correlation between the numbers of the various organisms discharged and the factors which might have a bearing on these are collected in Table 6. If the discharge were simply a mechanical thing one would expect correlation with the solids in the effluent, but this is found only for

Lumbricillus lineatus worms and cocoons, and *Metriocnemus longitarsus* larvae and pupae, and neither the worms nor these larvae are likely to be dislodged involuntarily from the beds. One would also expect correlation with the indications of abundance of the organisms, and this is generally found, but again *M. longitarsus* behaves exceptionally, as the numbers strained fail to correlate either with the emerging flies or with the larvae in the bed. The most interesting correlation that appears is the negative coefficient between the amount of surface growth and the following: the larvae of both *Psychoda* and of *Metriocnemus*, the pupae of the latter, and *Lumbricillus lineatus* worms and cocoons. This correlation does not hold good for the pupae of *Psychoda*, nor for *Spaniotoma minima* larvae or pupae, and the discharge of the early stages of this last species seems to be solely associated with abundance. As regards the worms, the reasons for their seasonal passage out seem definite and were given above (p. 126).

TABLE 6. *Coefficients of correlation between organisms escaping in the bed effluent and possibly related factors. Correlation is shown by a coefficient between* 0·5 *and* 1·0 (*sign* + *or* −). *Indications of abundance on the surface are by trapped flies or worms in surface growth, in the depths by trapped larvae or worms*

Form strained	Surface growth	Solids in effluent	Indications of abundance		
			Surface	1 ft. deep	2 ft. 6 in. deep
S. minima: Larvae	+0·528	−0·354	+0·631	+0·574	+0·717
Pupae	+0·483	−0·345	+0·715	+0·370	+0·347
P. severini: Larvae	−0·526	+0·248	+0·899		
P. alternata: Larvae	−0·563	+0·196	+0·699	+0·377 (few)	+0·571
Psychoda spp.: Pupae	+0·289	+0·022	+0·775	—	+0·544
M. longitarsus: Larvae	−0·704	+0·819	−0·106	+0·437	Few
Pupae	−0·670	+0·950	−0·095		
L. lineatus: Worms	−0·606	+0·626	−0·007	+0·795	+0·684
Cocoons	−0·507	+0·720	−0·242	+0·819	+0·838

When their food becomes scarce near the surface they descend in search of it. Heavy concentrations have then been detected in the depths, and it is supposed that this downward journey brings some of them to the floor of the bed where they easily pass out, still searching for food. It is likely that the same force is responsible for the increase of the larvae in the effluent (*S. minima* excluded), since it is reasonable to suppose that as the food drifts down the bed in the off-loading they would move with it, while the pupae would naturally be produced lower in the bed in consequence. There is negative evidence of this in two cases. *Metriocnemus longitarsus* larvae were very abundant in the top food bag from 16 October to 30 December; surface growth was abundant; only two larvae and one pupa were collected in the strainings during this period. Again, there was a great concentration of *Psychoda alternata* larvae at the lower food bag from 23 July to 7 October at a time when surface growth was increasing and 241 larvae were taken in eleven strainings, a small number compared with those strained out in spring when surface growth was becoming more scanty. As no concentrations of *P. severini* larvae were detected, its behaviour cannot be examined in this way.

The cocoons of *Lumbricillus lineatus* were present in very small numbers in the strainings considering their abundance in the beds, and they were particularly scanty in the cooler months when they are most freely produced. They are fixed at deposition, and the loosening of some at the off-loading is to be expected. When the worms are low in the beds the cocoons

will be deposited nearer the points of escape. The pupae of *Psychoda* are subject to a similar incidence in the strainings, and the numbers recorded bear a reasonable relationship to the number of larvae, young larvae being missed. The pupae of *Metriocnemus longitarsus* are relatively and those of *Spaniotoma minima* actually (and still more relatively) more abundant than their larvae. Their cocoons, especially that of *S. minima* which is made of debris, are readily attacked and, when this occurs, the active pupae wriggle free and have then little prehensile power. These pupae, however, often leave their cocoons voluntarily just before emergence, and so might be washed down if the distributor happened to pass when they were in this brief free condition. Therefore, their abundance relative to larvae in the strainings may be due not entirely to interference though it is probably due largely to this cause.

It is considered that a voluntary migration out of the bed is primarily responsible for the conveyance of larvae and worms into the effluent gullies rather than a mechanical force. If the organisms reach the floor of the bed and find food scanty it seems natural that they should pass through the floor into the channels which contain some humus. As the distributor passes along its traverse these channels receive a flush of water in succession, and this gathers speed as it flows to the end gully by way of the collecting channel. The flow in the channels would be likely to flush out *Psychoda* larvae, not adapted to clinging, and *Lumbricillus lineatus* with its habit of coiling round fragments of debris (Welch, 1914), also the cocoons and pupae. This action is much less likely with *Metriocnemus* and *Spaniotoma minima* larvae with their strong power of resistance. On these views the seasonal increase of the organisms in the bed effluent is to be understood.

It is not clear why *Psychoda severini* should suffer more in this competition than does *P. alternata*. At summer temperatures it is rather slower in development, especially in the egg and pupa stages, taking about one day longer in each, and as these stages are most liable to attack this may be a serious disadvantage. On the other hand, being parthenogenetic, *P. severini* has more breeding offspring and there is no loss through failure to mate such as might occur in *P. alternata*. At Huddersfield, W. H. Golightly finds the larvae of both species congregated in the top 1 ft. of the bed, and he thinks that the disadvantage that *P. severini* suffers may be due to the more sluggish habits of its larvae. These are more sclerotized and spinous than those of *P. alternata* which are vermiform and flexible. At Knostrop the larvae of *P. severini* did not enter the sunk food bags, and no zonal concentration has been detected there but it may be that addiction to the topmost 1 ft., together with a less flexible habit and a slower growth in summer, would make it a more ready victim to *Metriocnemus*. It is its almost total summer-autumn eclipse that is difficult to understand, since the species remains abundant at Barnsley under similar climatic conditions.

These studies on the fauna of the sewage bacteria beds have more than a theoretical interest. The beds become choked and useless unless the scouring organisms keep pace with the tendency for the growth and debris to accumulate. Once a bed shows signs of choking the wise manager rests it if possible, and the scourers have a chance to regain control. But in many small towns the beds are barely adequate to meet their demands and the sewage must go on or be discharged untreated into the rivers. If the sewage is still fed to the choking bed, it becomes foul and unsuitable to the scourers, and in the final stage becomes quite impervious. No scourers can then reopen it, but the cost of digging out the great mass of medium is prohibitive.

Beds are kept open by various combinations of scouring organisms. At the Huddersfield Works there is a fauna only of *Psychoda* spp., *Spathiophora hydromyzina* and some enchytraeids. At Barnsley to these are added abundant *S. minima*, and *Achorutes viaticus*. At Knostrop there is a varied fauna, *Metriocnemus* spp., *Lumbricus rubellus* and molluscs with swarming enchytraeids being added to the above, while there is no *Achorutes viaticus*. *Psychoda* spp. are well known to form an aerial nuisance near sewage works, and they most abound where the fauna is least varied. At Knostrop with its mixed fauna competition prevents any of the flies breeding to excess, while the beds are always open and never really require rest beyond the night hours when the flow is reduced and rest is convenient. There is no doubt that the mixed fauna is best for the beds, but the reasons for limitation are by no means clear, and only prolonged investigation by biologists can discover whether the secret lie in the character of the sewage, the routine of feeding it to the bed, the type of distributor, the medium used, or the way in which the bed is constructed.

Another practical aspect of these organisms concerns their metabolism. Are their excretory products more desirable material than the debris they destroy? Bell (1913) conducted experiments which showed that a given weight of "*Podura*" (*Achorutes viaticus*) in sewage material gave rise to a definite weight of ammonia and carbon dioxide, and suggested that these organisms had more than a mechanical function in the beds. This aspect of activity has more recently been referred to by Reynoldson (1939*a*) after a study of *Lumbriculus lineatus* in model beds.

The theoretical interest of the study of this fauna is diverse. Its capacity to form a basis for a study in competition amongst animals will be readily understood from the foregoing account. The occurrence of an organism at one works forming almost the entire scouring fauna and at another being just a unit in a mixed fauna gives an opportunity to compare the effects of intraspecific and interspecific competition. This aspect is now under investigation. The limitations of the fauna are also of interest. For instance, certain *Limosina* spp. (Borboridae) breed in the beds but not abundantly. Is the habitat not fully suited to them or are they kept down by more successful forms? Some Hydrophilidae and Staphylinidae of the Coleoptera are in the same case. The limiting factor that keeps *Spaniotoma perennis* from becoming successful denizen in open beds was mentioned above (p. 136). The bacteria beds in fact afford one more illustration of the saying that in a given habitat few insect species achieve abundance (Graham, 1933), and they seem to offer an opportunity of discovering why this is so in one type of environment.

The bacteria beds may also supply material for the study of problems of natural selection in another way. About fifty years ago it was realized that it was impracticable to filter sewage and the first beds of open medium were constructed (Barwise, 1899). Since then they have been placed in many spots and lands, though the more modern methods of treating sewage by the activated sludge process tends to slow down their further construction. Lloyd (1933) has pointed out their novelty as an insect habitat and those features that tend to isolate the fauna from more naturally dwelling forms. Such a factor is a degree of seasonal isolation due to earlier and more prolonged emergence of the insects bred in the warm beds. Another is the great extent of many sewage works and the density of the output of the successful flies, factors which together make inbreeding almost sure. Evidence is accumulating that selection in insects may be much more rapid than would have been believed possible a few years ago. For instance, fumigation in orchards seems to be producing strains of scale insects resistant

to this treatment (Dobzhansky, 1937). From such points of view the insect population of the bacteria beds is well worth observation and record.

Summary

Biometric observations at the Knostrop (Leeds) Sewage Works have been continuous for more than five years. The fauna in these bacteria beds is a balanced one and comprises in particular *Lumbricillus lineatus* (Enchytraeidae), *Psychoda* spp. and the chironomids *Metriocnemus* spp. and *Spaniotoma minima*.

The seasonal abundance of *Psychoda* spp. is shown to depart from what climatic influences would permit, especially as regards their abundance in late summer. Greater density of these flies is recorded from neighbouring sewage works where the chironomid fauna is absent or more limited than at Knostrop. The sweeping action of the Knostrop sewage distributors determines to some extent the relative abundance of *Psychoda* and chironomids in various parts of the beds, and a tendency for the latter to keep down the *Psychoda* is demonstrated. *Metriocnemus* larvae and *Lumbricillus lineatus* occupy the topmost foot of the medium, while *Psychoda alternata* larvae are found in greatest density below this zone. But the topmost foot is also the preferred zone of *Psychoda* larvae and they occupy it at neighbouring works where *Metriocnemus* is absent. On these several grounds a competition amongst the scouring organisms is evident and this should be capable of analysis.

The chironomid larvae in particular develop a predatory tendency in the absence of more normal food, and this is believed to be a strong element in the competition. Eggs, pupae and the worm cocoons are loosened from the medium by the activities of the grazing organisms, and it is shown that the numbers washed away from the beds in the effluent may be in part due to such interference. Most of the loss of larvae by this means, however, is associated with the spring off-loading of the beds and is due to the worms and larvae following the food they have loosened to the bottom of the bed and passing through the floor in search of nutriment. At this time scouring reduces the available food to small proportions and the fly breeding is checked in consequence. Over-population of the beds may also occur locally at other seasons of the year, and evidence is given of a consequent increased mortality in *Metriocnemus longitarsus* and a reduction in weight of the emerging flies of this species and of *Spaniotoma minima*.

The practical and theoretical interest of these studies is emphasized. While a number of organisms can as individual species do the essential scouring of the medium a balanced fauna is most advantageous. Competition prevents any one insect breeding to excess, and so fly annoyance is reduced.

Our thanks are due to the Managers of the Sewage Works where these investigations have been carried out, especially to Mr J. T. Thompson of Knostrop and to Mr H. D. Bell and his Assistant, Mr C. Wildsmith of Barnsley; also for identifications to the Director of the Imperial Institute of Entomology, to Dr F. W. Edwards, to Mr C. C. A. Monro, and to Dr W. S. Bristowe. We also express our gratitude to Prof. E. A. Spaul for every help that a departmental Head can give.

REFERENCES

Barwise, S. (1899). *Purification of Sewage.* London.
Bell, H. D. (1913). *Ann. Rep. Stratford-on-Avon Sewage Disposal Works.*
Dobzhansky, T. (1937). *Genetics and the Origin of Species.* New York.
Bristowe, W. S. (1939). *The Comity of Spiders. Roy. Soc.*
Dyson, J. E. B. & Lloyd, Ll. (1936). *Proc. Leeds phil. lit. Soc.* **3**, 174.
Fair, G. M. (1934). *Sewage Wks J.* **6**, 966.
Graham, S. A. (1933). *Ann. ent. Soc. Amer.* **26**, 499.
Johnson, J. W. H. (1914). *J. econ. Biol.* **9**, 105.
Lackey, J. B. (1924, 1925). *Bull. N.J. agric. Exp. Sta.* nos. 403, 407.
Ladell, W. R. S. (1936). *Ann. appl. Biol.* **23**, 862.
Lloyd, Ll. (1935). *Proc. R. ent. Soc. Lond.* **10**, 34.
—— (1937). *J. Inst. Sewage Purif.* Pt. 1.
Lloyd, Ll. & Turner, J. N. (1936). *Proc. Leeds phil. lit. Soc.* **3**, 177.
Meiklejohn, J. (1932). *Ann. appl. Biol.* **19**, 584.
Reynoldson, T. B. (1939). *Ann. appl. Biol.* **26**, 138.
—— (1939*a*). *Sewage Purif.* **1**, 72.
—— (1939*b*). *Ann. appl. Biol.* **26**, 782.
del Rosario, F. (1936). *Philipp. J. Sci.* **59**, 85.
Tonnoir, A. (1922). *Ann. Soc. ent. Belg.* **62**, 49.
Uvarov, B. P. (1931). *Trans. Ent. Soc. Lond.* **79**, 19.
Welch, P. S. (1914). *Bull. Ill. Lab. nat. Hist.* no. 10.
Woods, H. M. & Russell, W. T. (1931). *An Introduction to Medical Statistics.* London.

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THE ECOLOGY OF ACTIVATED SLUDGE IN RELATION TO ITS PROPERTIES AND THE ISOLATION OF A SPECIFIC SOLUBLE SUBSTANCE FROM THE PURIFIED EFFLUENT

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(With Plate 6 and 1 Text-figure.)

1. Development of activated sludge from free-living organisms

Activated sludge can be described as the sediment of micro-organisms and their debris obtained by long, continuous aeration of a nutrient medium containing free-living micro-organisms. Any kind of non-poisonous organic matter in solution, provided it contains the elements essential for growth will, if exposed freely to aerial infection, produce a sediment of micro-organisms on prolonged aeration. On continuous aeration and repeated renewal of the nutrient solution, the sediment increases in quantity and "settle-ability", and acquires the power of clarifying and also of removing organic matter from solution. The optimum power of the sediment (or sludge) for this purpose is obtained at temperatures between 25–35° C. and with a nutrient solution having an initial B.O.D.* not greater than about 75 parts per 100,000 equivalent to a solution of about 0·09% peptone or 0·12% dextrose.

The bacterial flora of such a sludge has not yet been fully investigated, probably on account of the difficulty of confirming the identity of the growths in pure culture with those occurring in the sludge. The taxonomy of the filamentous and zoogloeal forms of bacteria is still obscure. Such difficulties in identification are not experienced with the fungi and Protozoa which can be identified *in situ* with a reasonable degree of certainty.

Thus, Buswell & Long (1923), as the result of daily microscopic observations on the development of activated sludge from raw sewage, record a definite succession of species of Protozoa and bacterial forms. Lockett (1928) has listed the Protozoa found in activated sludge at Manchester, and arranged them in four groups according as the condition of the sludge is good, satisfactory, unsatisfactory, or bad.

2. Organisms occurring in activated sludge

The initial population of micro-organisms in a freshly exposed culture medium is varied and complex, but when an effective sludge has been obtained, the population consists of relatively few types which remain constant: e.g. peritrichous ciliates (*Vorticella*, *Carchesium*), *Aspidisca costata*, and rotifers with colonial forms of bacteria, but little or no fungi. Whenever a successful activated sludge has been produced, whether from sewage of an industrial city like Manchester or Milwaukee, from the domestic sewage of an Indian town like Bangalore, or from milk factory effluent, the protozoan fauna is found to be the same.

* B.O.D. is the abbreviation for Biochemical Oxygen Demand which is the American equivalent of the standard incubation test of the Royal Commission on Sewage Disposal, and is a very convenient method of determining the concentration of a nutrient solution. It is expressed in parts by weight of oxygen per 100,000, since the solubility of oxygen in water at 12° C. is 1 part per 100,000.

A comparison of the types of organisms in a freely aerated culture medium after short and long periods of aeration is given in Table 1. All the species mentioned may not occur at the same time owing to competition and the vagaries of chance inoculation. The transition from group A to B takes place gradually and is hastened at temperatures near 30° C. and with more dilute liquids of low B.O.D. (25–40). Sludges of either type are easily recognizable under the microscope. They can also be distinguished with the naked eye by their physical properties.

TABLE 1. *Organisms present in an aerated culture medium of 0·5 % milk in water, freely exposed*

A. During first 7 days of aeration	B. After aeration for 2 months
Fungi	
Fusarium	None
Penicillium	
Monilia	
Yeasts	
Bacteria	
Filamentous forms	Non-motile and
rods } motile and	dendroid colonies
cocci } non-motile	*Zoogloea ramigera*
Zoogloea ramigera	*Staphylococci*
Protozoa	
Amoeba limax	*Aspidisca*
Cochliopodium	*Epistylis*
Heteromita globosa	*Carchesium*
Bodo saltans	*Vorticella*
Oicomonas termo	
Cercomonas crassicauda	
Euglena	
Peranema	
Chilodon	
Colpidium	
Cinetochilum	
Stylonichia	
Pleuronema	
Glaucoma	
Lionotus	
Metazoa	
Eelworms	Eelworms
	Rotifers

3. PROPERTIES OF DIFFERENT TYPES OF SLUDGE

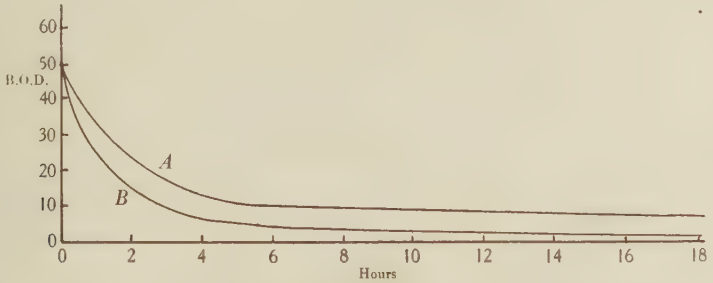
Sludge of type A has poor "settle-ability" and leaves the supernatant liquid more or less opalescent, imparting this opalescence also to successive washings of clear water. A sludge consisting of bacteria liable to dispersion, and therefore having more surface area, is less effective in removing organic matter from solution than one consisting only of bacterial zoogloea. Sludge of type B settles rapidly, leaving a clear sparkling supernatant liquid of very low B.O.D. (1 part per 100,000).

When the sludge is aerated for 10 days or more without the addition of any nutrient, the number of Protozoa diminishes, until no active forms remain. This is followed by a breaking up of the bacterial flocs; the protozoa seem unable to feed on bacterial flocs alone. This also applies to the eelworms and rotifers, and suggests that the fauna (ciliates and rotifers) takes

an active part in the direct removal of organic matter from solution and does not live chiefly by ingestion of bacteria. The absence of rhizopods from efficient sludge is noteworthy in this respect. They are known to feed almost exclusively on solid particles and are probably dependent upon solitary bacteria which do not flourish in sludge of type B. Cramer (1931) found that Protozoa are necessary to obtain a proper clarification of the supernatant liquid, but he did not confine this property to organisms in group B.

Conversely, when an efficient sludge is fed with a solution of too high a concentration (e.g. B.O.D. of 150–200) the micro-flora and fauna change owing to the development of other species, and the activity of the sludge is impaired (Barritt, 1936).

The rates of removal of organic matter from solution are also different for the two types of sludge as shown in Text-fig. 1.



Text-fig. 1. Rate of reduction of B.O.D. with two types of sludge.

Both types of sludge show a rapid initial reduction of B.O.D. Type B brings the reduction practically to completion in about 6 hr. whilst type A leaves a considerable residual B.O.D. unchanged after 24 hr. or more aeration. This residual B.O.D. is not intrinsically more difficult to remove since it readily disappears on contact with sludge of type B.

The rapid initial reduction of B.O.D. of the liquid can also be brought about by shaking the sludge and liquid in the absence of air. The results obtained by shaking different nutrient solutions in stoppered bottles with 15% activated sludge at 16° C. are given in Table 2 together with the results of open aeration treatment.

TABLE 2. *Reduction of* B.O.D. *of liquids by shaking with and by aeration with activated sludge*

		B.O.D. in parts per 100,000			
				Period of treatment	
Liquid	Treatment	Before mixing	After mixing	1 hr.	4 hr.
0·5 milk	Shaking	55	25	12	4·2
in water	Aeration	55	25	10	3·5
Lactose,	Shaking	51	42	28	12·0
0·075%	Aeration	51	42	13	1·6
Sucrose,	Shaking	50	38	11·5	8·5
0·075%	Aeration	50	38	8·0	1·4
Peptone,	Shaking	28	23	18·0	8·5
0·03%	Aeration	28	23	16·0	4·0

(*p*H 7·5)

Almost as much organic matter is removed from solution by shaking as by aeration. As in the case of more highly organized animals intake of food does not involve its immediate oxidation. Precipitation or adsorption must necessarily precede oxidation since the oxidative system is not in solution but confined to the cells of the organisms. Long-continued absence of oxygen from the sludge destroys its adsorptive power and may result in the liberation of the adsorbed matter into the solution again, probably due to anaerobic respiration.

This was shown when samples of the mixture from an experiment on the treatment of 0·5% milk in water with 15% (by volume) of activated sludge were removed at intervals. Each sample was divided into two halves, one of which was used for determination of B.O.D. immediately. The other half was incubated without aeration at 28° C. for 20 hr. and the B.O.D. of the settled liquid subsequently determined. (Table 3).

Prolonged incubation for several days without aeration results in autolysis and breakdown of the sludge.

TABLE 3. *Effect of anaerobic incubation with activated sludge on the* B.O.D. *of the paper filtered effluent*

	Period of aeration (hr.)		
Sample ...	3	5	24
Without incubation	6	3·5	1·5
After incubation	22·5	19·2	10·2
Increase in B.O.D. due to re-solution of absorbed milk	16·5	15·7	8·7

4. BACTERIAL COLONIES IN ACTIVATED SLUDGE

Sludge B has a much less complex bacterial flora than that of type A. It consists of flocs of apparently single cultures, mostly of the staphylococcus type (see Pl. 6, fig. 2).

Other types of bacteria may occur but they develop as pure colonies held together by a gelatinous matrix, the so-called *Zoogloea ramigera* (see Pl. 6, figs. 3, 5). The production of this gelatinous matrix is independent of the original composition of the nutrient solution and is not stimulated by the addition of sugar. Attempts by the plate method to isolate bacterial strains exhibiting similar types of growth in pure culture have so far failed. All the isolations grew in liquid culture with the usual dispersal and without any gelatinous matrix. Butterfield (1935) has described a zoogloea-forming bacterium isolated from activated sludge which he identifies with *Zoogloea ramigera*.

Analogy may be drawn with the "infection" threads of *Bacterium radicicola*, which are not produced in pure culture but only in the cells of its host. The thread prevents dispersal of the bacteria and general infection of the tissues of the plant.

In attempts to isolate strains by the dilution method in liquid culture, success was obtained with a strain of staphylococcus. It grew readily in 0·5% peptone-Lemco solution producing the typical flocs shown in activated sludge, and it readily forms a sediment. It does not grow in a medium consisting of broth made from the sludge itself. No purification experiments have yet been carried out with this organism in pure culture.

5. The isolation of the residual substance present in well-purified effluents

The rapid and almost complete removal of organic matter from solution appears to depend upon the association of the bacterial flocs and protozoa. The small residual amount of organic matter in the finished effluent is indicated by the oxygen absorbed from permanganate (4 hr. oxygen absorption test) and the oxygen uptake from water (5 days' B.O.D. test). It frequently happens that the permanganate test gives a higher figure (e.g. 1·3) than the B.O.D. test (e.g. 0·8) showing the presence of some compound which can more readily be oxidized chemically than biologically. In the case of crude sewage the reverse is always true (e.g. 15 Ox. abs. and 25 B.O.D.).

The stability of viruses of the tobacco mosaic type, to enzyme action, suggests a similarity to the residual organic matter of the purified effluent and the possible use of activated sludge as a means of removing extraneous organic matter from crude virus preparations. Bawden (private communication) has found these viruses to survive aeration with activated sludge, but did not find the sludge effective in purifying the virus by the method tried. It therefore seemed possible that compounds similar to viruses might survive the sewage purification process and account for the residual organic matter occurring in a purified effluent.

Virus preparations of aucuba disease of tomato obtained from Bawden were submitted to the permanganate and B.O.D. tests respectively with the following results:

Oxygen uptake of 0·3 % *virus preparation, in parts per* 100,000

From permanganate (4 hr. at 27° C.)	22·5
B.O.D. (5 days at 20° C.)	16·5

These results are similar to those obtained on the purified sewage effluent showing a higher oxygen absorption from permanganate than from dissolved oxygen.

The clear purified effluent from an activated sludge tank treating 0·5 % milk in water was submitted to the isolation technique employed with plant virus. A slight flocculent precipitate was obtained on standing after the addition of 20 % ammonium sulphate to the effluent. On purification of this precipitate by repeated reprecipitation and centrifuging, a viscous solution was obtained containing, after dialysis to remove ammonium salts, 0·2 % of the substance. This substance resembles tobacco mosaic virus in that it shows the phenomenon of anisotropy of flow when agitated between crossed nicols, indicating the presence of asymmetric particles. This property, however, is not destroyed by heating to 100° C. but is lost in the case of virus.

The purified effluent from the filter beds of the Harpenden sewage works yielded a substance with identical properties.

Analysis of the dried purified material showed 8·8 % organic nitrogen by micro-Kjeldahl, and 0·2 % phosphorus by Fiske & Subarrow's method. The substance gave negative tests with xanthoproteic and Millon's reagents, and did not reduce Fehling's solution after boiling with dilute mineral acid.

The precise biological significance of this material is at present unknown. It was found to have no infectivity for plants, and considering its wide distribution it is unlikely to possess any for animals.

The substance is also obtained by extraction of the sludge at 30° C. with water made alkaline with ammonia at *p*H 8·0, and is probably a specific water soluble substance of the zoogloeal bacteria of the sludge.

Summary

1. The formation and properties of activated sludge from a miscellaneous growth of free-living organisms is described.

2. The properties of the sludge are dependent upon definite groups of organisms which appear to be mutually selective.

3. Using the Royal Commission test of biochemical oxygen demand as a measure of concentration of nutrients in solution, it is shown that micro-organisms take up nutrient independently of its oxidation and that the unoxidized nutrient may be released from the cells in the continued absence of oxygen, probably due to anaerobic respiration.

4. In the case of some strains of bacteria, growth in the sludge occurs in the form of strands or colonies held together by a gelatinous matrix and resembling the "infection" threads of the *Bacterium radicicola*. The phenomenon is apparently not produced in pure culture.

5. A substance possessing anisotropy of flow when agitated between crossed nicols is shown to occur in a well-purified effluent, but differs from virus protein in being stable at 100° C.

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REFERENCES

Barritt, N. W. (1936). *Rep. 2nd Int. Congr. Microbiol.* Sec. 4.
Buswell & Long (1923). *J. Amer. Wat. Wks Ass.* **10**, 2.
Butterfield, C. T. (1935). *U.S. Treasury Dep. Publ. Hlth Rep.* **50**, 671.
Cramer, R. (1931). *J. industr. Engng Chem.* **23**, 309.
Lockett, W. T. (1928). *Ann. Rep. City Manchester Rivers Dep.* Appendix 1, p. 40.

EXPLANATION OF PLATE 6

Fig. 1. Sludge of type A showing various types of bacteria in dispersion.
Fig. 2. Sludge of type B showing single type of *Zoogloea*.
Fig. 3. *Zoogloea ramigera* in sludge.
Fig. 4. Colony of rods occurring in sludge.
Fig. 5. Colony in gelatinous matrix or "infection" thread in sludge. Note absence of dispersed bacteria in the liquid.
Fig. 6. Typical isolation from zoogloeal clumps showing dispersion.

(*Received* 8 *July* 1939)

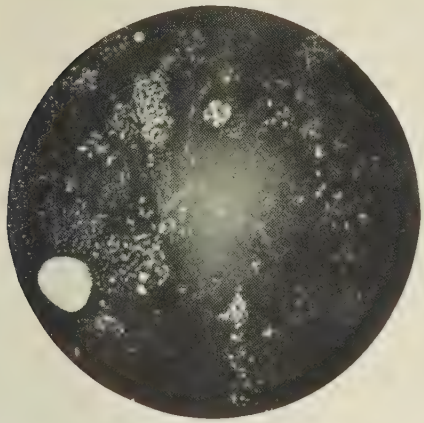

Fig. 1.

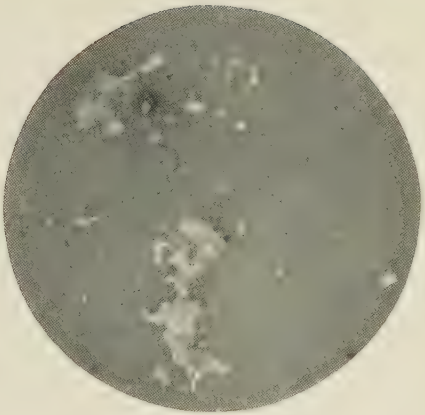

Fig. 2.

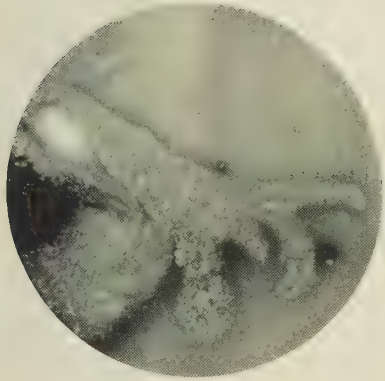

Fig. 3.

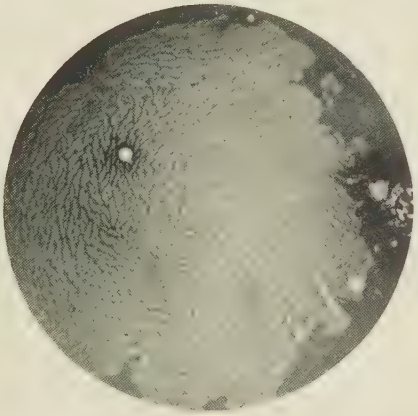

Fig. 4.

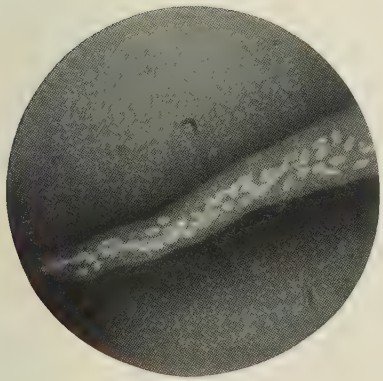

Fig. 5.

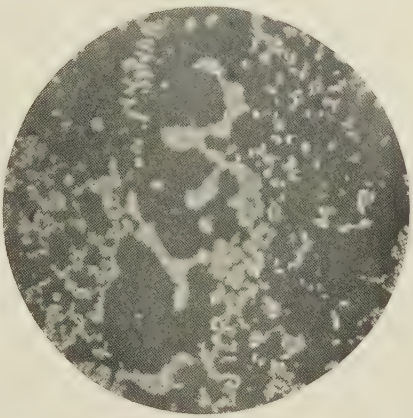

Fig. 6.

BARRITT.—The Ecology of Activated Sludge in Relation to its Properties and the Isolation of a Specific Soluble Substance from the Purified Effluent (pp. 151–156)

REVIEWS

Quantitative Zoology. By G. G. Simpson and A. Roe. Pp. xv+141, 52 figs., 13 tables. London: McGraw Hill Publishing Co., Ltd. 1939. 26*s*. 3*d*.

This book is intended to supply a sound mathematical basis for the worker in systematic or observational zoology. There are chapters on the basic principles of mensuration, on frequency distributions and their patterns, a good account of the properties and use of the so-called "normal" curve; discussions on sampling and the comparison of samples; on correlations, regressions and on contingency tables. Of special value to the systematist is the discussion on what can reliably be inferred from small samples and single specimens such as museum "types". The last chapter is on problems of growth rate, and there are in addition a glossary of mathematical terms, a summary of the principal symbols and formula, and several useful tables to assist in calculation of distributions and the significance of results.

From the statistical point of view one should note that the authors calculate the mean variance and standard deviation by dividing the total variance by the number of observations (n) and not by the number of degrees of freedom ($n-1$). They draw attention to this however, and point out that for small samples the latter is more accurate. They do not deal with analysis of variance, and although they discuss partial correlations at some length they do not mention partial regressions, which are in many ways more valuable to the Zoologist than correlations.

There is no doubt whatever that any Zoologist who has to deal with measurement and the comparison of measurements, will do better work and exercise a more critical judgement if he is familiar with arguments and processes to which this work is a very fair and readable introduction.

C. B. WILLIAMS

Elements of Plant Pathology. By I. E. Melhus and G. C. Kent. Pp. x+493. New York: The Macmillan Co. 1939. 21*s*. 0*d*.

The authors have attempted to arrange a course in plant disease on a pathological rather than a mycological basis, and suited to students wishing to "become better farmers, teachers or agriculturists" rather than disease specialists. The first quarter or so of the book deals with the generalities of the subject—importance of plant disease, symptomatology, parasitism, environmental relations, and principles of control; and this is followed by a consideration of specific diseases grouped according to the taxonomic position of the parasites.

The general chapters are well done and provide a sound basis, but there are matters which invite criticism. Thus in certain chapters there is a lack of perspective; e.g. Ch. 3 which almost conveys the impression that the development of plant pathology since about 1888 has taken place exclusively in the U.S.A. Here and there one finds a certain lack of balance in the subject matter; e.g. the chemical detail in Ch. 7 as compared with the elementary treatment of many other matters. From time to time there are textual passages which need emendation; e.g. on p. 83 one reads, "A fungicide applied to the upper surface of a leaf usually will prevent an attack on the lower surface" which is followed immediately by advice to apply fungicides so that "a continuous film covers all the surfaces of the susceptible parts of the host". Further, throughout these chapters the text is clogged, irritatingly and quite unnecessarily, by names having full taxonomic reference; e.g. "*Fusarium bulbigenum* Cke. and Mass. var *lycopersici* (Brushi.) Woll. and Rg."

In dealing with specific diseases the authors commence with those caused by Phycomycetes, then surprisingly pass to bacteria, and viruses, and then return to fungal diseases caused by Ascomycetes, Fungi Imperfecti, and Basidiomycetes. The authors explain that these chapters have been arranged "not according to their systemic positions, but in a manner that has proved to facilitate a more rapid understanding of parasitism". This may be the authors' experience but there is nothing in the text to justify it, and one rather imagines that its rationale lies more in the personal exposition of the authors than in the subject arrangement of the teaching.

Of the arrangement within the chapters the authors state that it "is based on similarity of patho-

logical changes that facilitates the student's understanding of disease processes". Again in the authors' experience this may be so, but its rationale is not obvious. In Ch. 13, for example, it is difficult to see any convincing reason for the sandwiching of onion smut between the oat smuts and the wheat smuts, or in Ch. 11 for the following particular order of consideration—chestnut blight, brown rot of stone fruits, lettuce drop, *Thielavia* root rot of tobacco, ergot of rye, alfalfa leaf spot, apple scab, powdery mildew of the grape, powdery mildew of barley, peach leaf curl, and *Graphium* wilt of elm.

Of the subject material the authors state: "The diseases chosen as types have been selected because of their illustration of certain principles or practices, because of their relation to the development of the science of plant pathology or because of their importance in agriculture." "...the material in the text is of significance to most agricultural regions and illustrates the principles fundamental to the subject." "The local importance of certain diseases may call for additions to or substitutions for the material presented." Even for many wide regions of N. America there would need to be very considerable addition and substitution, and students in this country will find little or no mention of at least one-half of our serious plant diseases. Thus Ch. 12 on diseases caused by the Fungi Imperfecti, includes only *Diplodia* dry rot of corn, *Phymatotrichum* root rot of cotton, *Cercospora* leaf spot of the beet, and the *Fusarium* wilts of water-melon and cotton: and Ch. 10 on virus diseases of plants, after 24 pages of general matter, deals only with common tobacco mosaic, sugar-cane mosaic, curly top of sugar beet, and aster yellows.

The authors follow the usual American custom of including short chapters on diseases caused by parasitic seed plants and by nematodes. A final chapter dealing briefly with diseases caused by non-parasitic agents includes only bitter pit, scald, and graft-knot of apple, and boron deficiency. In view of the increasing recognition of the widespread importance of this class of diseases this treatment might appear to be inadequate.

In all these chapters the consideration of individual diseases is well done and, on the whole, the authors have succeeded in minimizing the mycological aspects of pathology and emphasizing the phenomena of parasitism in disease processes. Again, however, there is plenty of scope for cavil. Thus Text-figs. 7 and 23 and the numerous diagrams of host relationships seem to me just a waste of space. Here and there the nomenclature is not up-to-date. Of *Rhizopus* the authors state: "...the pathogen grows to the surface and forms a thick bushy mass of mycelium and conidiophores bearing the large black conidia.... Conidiophores...bear at their tips large black conidia filled with the asexual spores. These spores are liberated by the bursting of the conidium...." This kind of thing just makes nonsense of scientific terminology and, moreover, is inconsistent with the glossary where the terms are correctly defined.

The glossary definitions are sometimes clumsy or such as may not be generally acceptable. Most pathologists use the terms "disease" and "malady" interchangeably, but the authors define "disease" as "The sum of the deviations of the vital functions beyond the latitude of health", which is clumsy, and "malady" as "A multiplicity of diseased individuals", which is inaccurate.

The bibliography is headed: "A list of books dealing with some phase of plant pathology" and a footnote reads "As far as possible these books might be made available to the student for reference reading." One expects, therefore, carefully selected works which together cover the general field of plant disease and which, individually, guide the student to more specialized regions. What one actually finds is a dreary jumble of text-books, ancient and modern, good, indifferent and bad, trivial and advanced, even old and new editions cheek by jowl cited as separate works. I have criticized this book freely largely because I consider it to be a serious contribution to the teaching of plant pathology: the blemishes noted may easily be dealt with in a second edition. An excellent feature of the book are the numerous illustrations, many of which are from original photographs. There is a good index and the book is rather beautifully produced.

W. B. BRIERLEY

Experiments in Plant Physiology. A Laboratory Textbook. By W. E. Loomis and C. A. Shull. Pp. xiv+213, 52 figs. London: McGraw-Hill Publishing Co., Ltd. 1939. 12*s.* net.

This book is a revision, partly rewritten, of the same authors' *Methods in Plant Physiology*. It is intended to be used as a classroom manual, but will well repay reading for its own sake by any student. The experiments are classified as elementary or more advanced, the latter requiring either more skill or

more attention than can normally be given in a large elementary class. The experiments are excellently devised and make a very welcome change from the usual stock ones of the physiology classroom. It is especially refreshing to find experiments included on growth differentiation and correlation. After each experiment is given a list of questions designed to make students think out for themselves the implications of their results. A brief list of useful and well-selected references is added to each section. Altogether an invaluable book. R. H. STOUGHTON

Seed and Potting Composts: with Special Reference to Soil Sterilization. By W. J. C. LAWRENCE and J. NEWELL. Pp. 128, with a Frontispiece and 20 figures. London: Allen and Unwin, Ltd. 1939. 3*s.* 6*d.*

The authors state: "This is not a book about all kinds of composts but about two kinds of composts for all kinds of plants. These composts are the outcome of experiments to meet the practical needs of cultivation at the John Innes Horticultural Institution." In a foreword Sir Daniel Hall writes: "The experiments ran into thousands; each step was made secure before going on a stage, until in the end they have arrived at a couple of composts which fulfil the definition I have given of being scientific—they will give the same results every time they are tried. They are not only standard composts but I have every reason to believe they are optimum composts which will give as far as the soil goes the best possible results". Owing to the admirable way in which they have been demonstrated at various shows these composts are now widely known and used, and results are rapidly confirming the claims made for them.

This book describes the rationale underlying the preparation and use of the John Innes standardized seed and potting composts. It is written so clearly and all necessary practical details are given so explicitly that there can be no excuse for any grower failing to benefit by its reading. Sir Daniel Hall states: "I regard their work as one of the most exact and valuable pieces of science applied to horticulture that has fallen within my experience", and there will be very few knowledgeable people indeed who will differ from this conclusion. Everyone interested in the growing of plants should buy and read this book. W. B. BRIERLEY

Practical Plant Breeding. By W. J. C. LAWRENCE. 2nd edition. Pp. 155 with 34 illustrations. London: Allen and Unwin, Ltd. 1939. 5*s.* 6*d.*

In this new edition of "the best introduction I know to plant genetics" (see *Ann. appl. Biol.* 1938, **25**, 224) misprints have been corrected, a few paragraphs made clearer, heat treatment to induce chromosome doubling replaced by colchicine treatment, the bibliography brought more but not quite up-to-date, and the technical equivalents of five common names inserted on p. 152. W. B. BRIERLEY

Recent Advances in Plant Genetics. By F. W. SANSOME and J. PHILP. 2nd edition, revised and rewritten by F. W. SANSOME. Pp. xii+412. London: J. and A. Churchill, Ltd. 1939. 18*s.* 0*d.*

The first edition of this book appeared in 1932 (see *Ann. appl. Biol.* 1934, **21**, 173). The new edition which has been revised and largely re-written by Dr Sansome alone, follows in general the lines of the earlier book but the first two chapters have been re-arranged, a great amount of new material has been incorporated, and a chapter on variegation and chimaeras has been added. The new edition also contains a useful subject key to the modern literature, and the bibliography which in the first edition occupied 47 pages now runs to 51 pages. Dr Sansome has performed his task most ably and the book is a first-class guide to the data and viewpoints of modern genetics. W. B. BRIERLEY

The Evolution of Genetic Systems. By C. D. DARLINGTON. Pp. xi + 149. Cambridge University Press. 1939. 10*s.* 6*d.*

For most of us the wealth of particular data in modern cytology is almost overwhelming; we realize that the individual facts are merely parts of a genetic mechanism, but we have little knowledge of that mechanism as an integrated and going concern, and still less appreciation of it as an evolved and evolving system. In this book the author has attempted to give just this philosophic understanding and evolutionary perspective.

No one reading the book carefully, and its extreme condensation makes careful reading imperative, can fail to be stimulated by the author's views or the brilliance of their exposition, although many readers may feel that he tends to be over-selective, is often a little too partial to his own interpretations, is here and there too dogmatic, and frequently does not give sufficiently adequate bibliographic reference for statements he makes.

Two years ago Dr Darlington published the second edition of his *Recent Advances in Cytology*, which was a masterly ordering of a huge mass of data. In this pithy book he has now analysed and integrated this material and, in a very remarkable way, has shown that all these complex phenomena are the natural developments of a few basic and relatively simple principles. The quality of this book and the importance and far reaching implications of the author's conclusions make the work a notable contribution to biological literature.

W. B. BRIERLEY